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EDITORIAL

ARTIFICIAL INSEMINATION

WE publish in this issue of the *Veterinary Journal* an interesting article on a neat method of practising artificial insemination in cattle. Artificial insemination as a method of breeding cattle is gradually being extended in many countries, including our own; work is also in progress to determine the most practical techniques likely to yield satisfactory results. Our knowledge of the whole subject has increased considerably since the early experiments were carried out, and as new methods are evolved we find them being used throughout the world. In considering artificial insemination, constant attention has to be given to a number of points, which include the evaluation of the fertility of the semen used for the work and the careful application of recognised techniques. It follows, therefore, that any new discovery has to be thoroughly tried out before it can be recommended as a practical measure. This means that research work both in the laboratory and in the field have to be followed by large-scale trials. In the equipment designed by Prof. Sorensen we have a simple method of inseminating which has been proved efficient and practical following its use throughout Denmark. Simplicity is often the keynote to successful application of methods and techniques and may go a long way in determining the practical value of some discovery. Whether this simple and ingenious method will be practised in countries other than Denmark remains to be seen, but it doubtless should be tried under the varying conditions found throughout the world.

Artificial insemination is gaining in popularity in different countries. While in this country it is the present custom to practice the operation in a fairly restricted area in the vicinity of a centre at which bulls are kept and semen is handled, it has been shown in America and elsewhere that semen properly prepared by dilution and properly preserved can be used over quite a wide area. In America, the usual practice is to establish large centres where many bulls are kept and at which the semen is handled. From these centres, semen for use is sent considerable distances in containers suitable to ensure that the temperature of the semen is maintained at a sufficiently low level to prevent its deterioration. By the adoption of this method it is possible to cater for large numbers of cows

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and heifers at long distances from the main centre. It is possible that an extension of areas served from centres in this country may also be made on lines somewhat similar to those in operation in America. But we must be satisfied that our conditions of transport will allow of such extensions. This can be known only by trials under controlled conditions.

We have still a mass of knowledge to acquire on the subject of artificial insemination. We know from experience that the present techniques are sound and yield satisfactory results; improvement in the techniques must be sought all the time. It is becoming more evident that our knowledge of sperm production in its various phases is by no means extensive, and research work on the subject must form part of our programmes in the general study of fertility and infertility. It seems evident that too little attention has been given to fertility studies in the male; efforts have been concentrated largely on the female, although here, again, we have much to learn. The whole subject of fertility and infertility in livestock will have to receive more attention from the research workers in the laboratory and in the field before we can go much further in the methods we are now adopting for its control. It can be claimed with some satisfaction that we in this country are practising control measures in dealing with infertility in animals to a greater extent than we did some years ago. Without, however, a basic knowledge of some of the essential features concerned with normal fertility and breeding, we find that we cannot progress far in many of the problems which confront us.

With a deeper knowledge of this subject, with the application of the results of research work as they come along, and with artificial insemination to assist us, we will eventually be in a happier position to control infertility and breeding operations to the benefit of the livestock industry and the country as a whole.

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GENERAL ARTICLES

A CASE OF DUPLICITAS IN A CALF

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THE subject to be described was the progeny of a Shorthorn cow and had been born dead, about six weeks prematurely. It appeared on superficial examination to possess two distinct heads and necks, four fore limbs, two hind limbs and two tails. Further duplication was evident in the region of the trunk (see photograph facing page 234).

It will be convenient to use the letters D (for Dexter) and S (for Sinister) to indicate what would be the right and left individuals respectively if the duplication had been more complete when the subject is viewed with the hind limbs in the normal relationship to the ground. In this position the spine of D ran along the top line of the body from head to tail. In the case of S the neck was bent sharply backwards and to the left, so that the head lay between the left fore limb of this individual and the common left hind limb. The spine of S was found to pass backwards from the neck across the left side of D, then, running mesially to the external angle of the left ilium, it led to the root of the left tail. The mid-ventral line of D, running forwards from the inguinal region between the two hind limbs, passed by the single umbilicus, then between the left fore limb of S and the right fore limb of D to reach the bases of the two necks, where it was continuous with a transversely running groove. From this groove the line passed backwards, as the mid-ventral line of S, between the two other forelimbs, then to the left of the spine of D to end at a slight eminence situated about six inches in front of the roots of the tails.

The right forelimbs of both D and S lay in the normal position in relation to the thorax, but the left limbs were bent outwards at the shoulder joints and the medial surfaces looked downwards. The limbs of D were slightly larger than those of S. The hind limbs met each other in the ordinary way in the inguinal region, but the quarters were separated from one another dorsally by the two distinct vertebral columns. They appeared to be otherwise normal and were comparable in size with the fore limbs of D.

The two heads were perfectly normal, but in dissecting the necks irregularities were noted towards their bases. The sternal attachments of the panniculus muscle ended in a fibrous aponeurosis lying on either side of a raphe, about three inches long, connecting the anterior extremities of the two sterna. This raphe was continuous with the mid-ventral lines of the two individuals and it gave origin to the sterno-cephalici and sterno-thyro-hyoidei muscles on either side of each neck. Beneath the sterno-thyro-hyoidei muscles lay the thymus glands. The brachio-cephalici muscles differed only in their direction beyond the shoulder joint in the two left forelimbs owing to the change in the position of these limbs already described. The muscles turned sharply outwards at the shoulder joints, each passing internal to the joint instead of over its anterior aspect to reach its

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insertion into the humerus. The pectoral muscles were entirely normal, as might be expected from the fact that there were two complete and distinct sterna. The thorax was represented by a large cavity which had resulted from a duplication of its walls. The left ribs of D met the right ribs of S at a sternum lying on the left side of the subject, and in a similar way the right ribs of D and the left ribs of S joined a sternum on the right of the subject. In the case of the abdomen the duplication was more pronounced. The ventral wall of the abdomen of D appeared normal, the muscles having attachments on the right and left sides to the right ribs of D and the left ribs of S respectively. The muscles of the abdominal wall of S were not so well developed. This was particularly noticeable posteriorly, where they merged in an aponeurosis in the region of the surface eminence already mentioned lying in front of and between the roots of the two tails. The muscles of right and left sides in this case were attached to the right and left ribs of S and D respectively.

The muscles of the hind limbs were generally normal, those of the quarters arising from the adjacent innominate bones and sacral spines, whilst inside the thighs there were the usual muscles taking origin from the ischio-pubic symphysis. The psoas muscles arose from the right and left sublumbar regions of D and S respectively. Between the roots of the two tails there was a small area in which was found a mass of small bundles of muscle, which were obviously the rudimentary muscles of the undeveloped right and left hindquarters of S and D respectively. A similar indeterminate mass of muscle lay above the entrance to the pelvis, with rudimentary psoas muscles from the left and right sides of D and S respectively running into it.

The two diaphragms had united to form a single curtain between the thoracic and abdominal cavities. The attachment of the muscular rims to the four series of ribs showed little deviation from the normal, and there were four phrenic nerves. Dorsal to what might be called the left sternum, since it lay on the left side of the subject, the diaphragm showed a large opening through which projected a rounded mass of the liver. The peritoneum and pleura, however, formed a complete septum between the abdominal and thoracic cavities. There were right and left foramina sinistra and hiatus aortici, but whilst the right foramen dextrum was large the left was very rudimentary. The lungs of S, i.e., the lungs lying beneath the spine of S, were considerably smaller than those of D, but apart from this these organs were quite normal.

The heart was single and relatively large. It was enclosed in a complete pericardium and extended across the roots of the necks, covering the entrance to the thoracic cavity, its base lying at the root of the neck of D and its apex at the root of that of S. Covering the pericardium were the two thymus glands, their bases meeting in the position of the fibrous raphe connecting the anterior extremities of the two sterna. The apex of the pericardium was attached posteriorly to the roots of the lungs of S, whilst sterno-pericardiac ligaments attached it on either side to the dorsal surfaces of the two sterna. What would normally be the left surface of the heart was directed ventrally, and this was crossed in the region of the ventricles by a deep groove which had been produced by pressure of the fibrous raphe mentioned above.

The aorta curved upwards and forwards to the spine of D, and after being

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joined by the ductus arteriosus it passed backwards on the left of the middle line of this individual, the œsophagus lying at first on its right side, then running below it to the foramen sinistrum. Passing through the diaphragm in the usual way, the aorta ran beneath the lumbar vertebræ as far as the entrance to the pelvis, where it turned sharply to the left to continue as the aorta of S. This vessel lay in the usual position at the roof of the left abdominal cavity, and traced forward was found to pass through the left hiatus aorticus and into the thorax of S. Reaching the roots of the lungs, it detached axillary and common carotid branches to supply the forepart of S, and then divided into two pulmonary arteries beneath the trachea. The arrangement of the main branches of the aortæ may be gathered from Fig. 1.

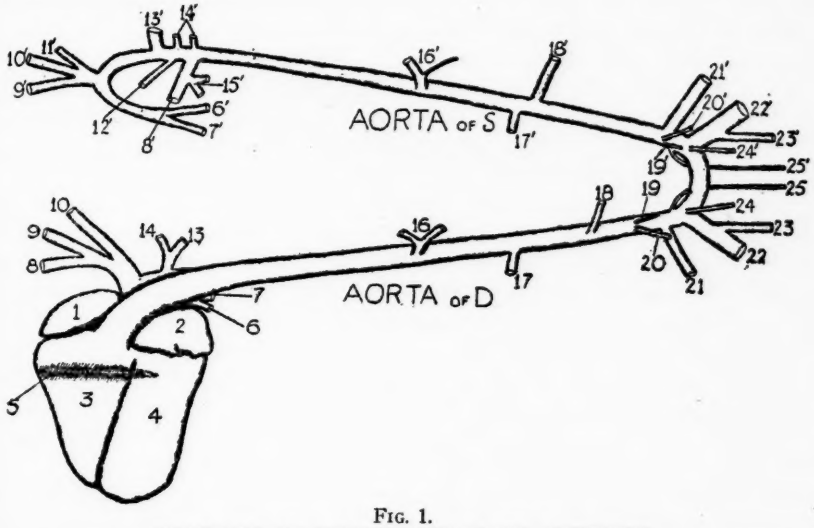

FIG. 1.
DIAGRAM OF HEART AND MAIN ARTERIES.
1.—Right auricle. 2.—Left auricle. 3.—Right ventricle. 4.—Left ventricle. 5.—Impression left by raphe at bases of necks. 6, 6′.—Left pulmonary a. 7, 7′.—Right pulmonary a. 8, 8′.—Right axillary a. 9, 9′.—Right common carotid a. 10, 10′.—Left common carotid a. 11′.—Left superior cervical a. 12′.—Right superior cervical a. 13, 13′.—Left axillary a. 14, 14′.—Left vertebral and subcostal aa. 15′.—Right vertebral and subcostal aa. 16, 16′.—Cœliac and anterior mesenteric aa. 17, 17′.—Right renal a. 18, 18′.—Left renal a. 19, 19′.—Dorsal utero-ovarian a. 20, 20′.—Ventral utero-ovarian a. 21, 21′.—External iliac a. 22, 22′.—Umbilical a. 23, 23′.—Internal iliac a. 24, 24′.—Ventral middle uterine a. 25, 25′.—Middle sacral a.

The pulmonary veins of D opened into the left auricle in the usual way. The innominate veins opened separately into the right auricle. The posterior vena cava on this side might be said to commence at the roof of the pelvic cavity, where it was formed by the union of right external and internal iliac veins, and a trunk resulting from the union of the left external and internal iliac veins, and the two renal veins of S. It pursued a normal course along the roof of the right abdomen, across the upper surface of the liver, through the right foramen dextrum and then, with the right phrenic nerve, between the diaphragmatic and accessory lobes of the right lung to open into the right auricle. It received the usual collateral tributaries.

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In S the innominate veins united with one another and also with the pulmonary veins of this side in a mass of indefinite tissue lying behind the apex of the pericardium. From here a large vein ran between the pericardium and the diaphragm to open into the right auricle at the opening of the coronary sinus. The left posterior vena cava in the abdominal cavity was represented by two trunks, the renal veins, lying on either side of the corresponding aorta. The vessels received small lumbar veins, and posteriorly they united with the posterior vena cava of D. A vestigial vessel led from the left umbilical fissure of the liver through the diaphragm to reach the apex of the pericardium, where it joined the pulmonary and innominate veins of S.

Each individual possessed a complete and distinct spinal cord, and only in the sacral region was there a deviation from the normal. The nerve supply of the hind limbs was derived from the right and left lumbo-sacral plexuses of D and S respectively. The inferior primary divisions of the left side of D and the right side of S in this region were very small, but they were seen to form a plexus from which nerves could be traced to the bundles of muscle lying at the roof of the pelvis. The superior primary divisions were similarly distributed to the muscles lying between the vertebral spines in this region.

The abdominal cavity consisted of two compartments continuous with one another through a large oval opening. The opening was bounded anteriorly by the common diaphragm, above and to the right by the vertebral column of S, and posteriorly by the left and right kidneys of D and S respectively. It was occupied by a portion of the single liver and, behind this, by coils of the left small intestine. The right compartment of the cavity was considerably larger than the left, for it contained, in addition to the normal viscera, the greater part of the common liver. The divisions of the stomach on this side were unusually arranged. The rumen was small, with the reticulum lying below and on either side of its anterior extremity. The omasum was situated below this, and the abomasum behind this again. After a length of about 12 feet the small intestine was joined by the intestine of the left side, the two bowels running closely applied to one another for some distance before uniting to form a single gut. Beyond this point the small bowel continued in the usual manner, lying below the spiral of the colon, to open into the cæcum. The large intestine pursued a normal course to the rectum, which opened at what might be described as a common cloaca. The right spleen was small, but attached to its upper extremity was an irregularly shaped accessory spleen. The right pancreas appeared normal in every respect.

The left compartment of the cavity was small and laterally compressed. It was occupied by the left extremity of the liver and, behind this, by the left stomach, spleen, pancreas, and the first 12 feet of the small intestine. With the exception of the liver, all these organs appeared to be normal. The small intestine passed into the right compartment and joined the right intestine as previously mentioned. The common liver (Fig. 2) was a large irregular mass lying for the greater part in the right cavity behind the diaphragm. Anteriorly a rounded lobe, cut off from the remainder of the organ by a deep groove, projected into the thorax through the opening in the diaphragm. The organ presented dorsal and ventral surfaces which were wholly in contact with the walls of the abdomen with the exception of the posterior part and left extremity of the dorsal surface, where

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the organ was related to viscera. Across the dorsal surface and near the centre ran a broad ridge, which was accommodated in the left inferior costo-vertebral groove of D. To the right of this, and running parallel to it, was the posterior vena cava of D. A fibrous cord representing the umbilical vein of S was seen to pass upwards from the floor of the abdomen of S in the falciform ligament to enter a second small left umbilical fissure. There were two portal fissures, one lying below the posterior vena cava, the other, much smaller, lying to the left of this. Two gall bladders were present, one near the larger portal fissure, the other near the right extremity of the organ. The umbilical vein, which led from the umbilicus in the right abdominal wall, joined the organ near its right and posterior extremity.

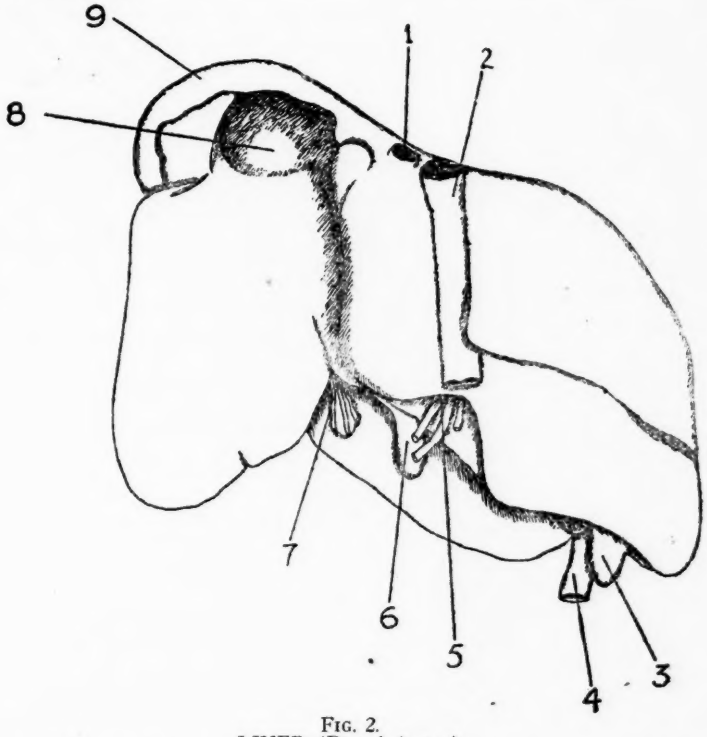

FIG. 2.
LIVER (Dorsal Aspect).
1.—Hepatic Vein. 2.—Posterior Vena Cava. 3.—Right Gall Bladder. 4.—Umbilical Vein. 5.—Right Portal Fissure. 6.—Left Gall Bladder. 7.—Left Portal Fissure. 8.—Depression for Left Reticulum. 9.—Lobe Projecting Through Diaphragm.

There was a complete duplication of the urino-genital system. Of the four kidneys, the right kidney of D and the left kidney of S were of normal size and they were situated in the usual position beneath the sublumbar muscles. The ureters from these two kidneys opened in the usual manner into a bladder, which lay on the floor of the pelvis. From its anterior end a urachus ran forwards to the umbilicus accompanied by umbilical arteries which were detached from the aorta as it ran across the entrance to the pelvic cavity. The other two kidneys were smaller and more irregular in shape. They were situated close together

just in front of the point where the two aortæ met. The slender ureters opened into a narrow tube about four inches long, which represented a bladder.

The subject could be said to possess but a single pelvic cavity, although in the roof of the cavity was a narrow passage, bounded on each side by the bodies of the sacral vertebræ, which was occupied by the rudimentary dorsal genitalia with vestiges of the muscles of the left and right hind limbs of D and S respectively. The single rectum opened at a prominent anus which was enclosed by thick folds continuous above and below with the labia bounding the two vulvæ. The larger vulva of the ventral genitalia appeared quite normal. The smaller

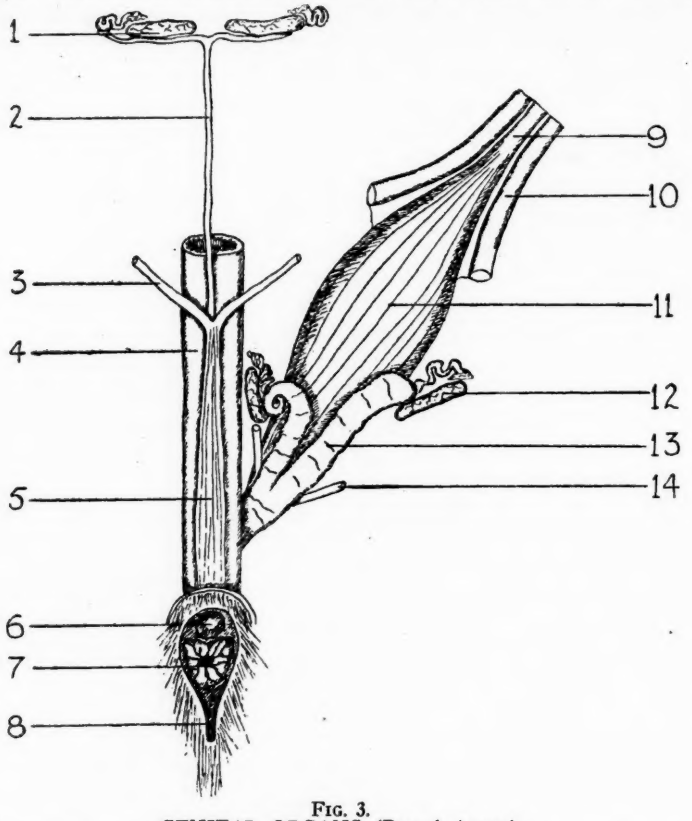

FIG. 3.
GENITAL ORGANS (Dorsal Aspect).
1.—Left Dorsal Ovary. 2.—Dorsal Uterus. 3.—Left Dorsal Ureter. 4.—Rectum. 5.—Dorsal Bladder. 6.—Clitoris in Dorsal Vulva. 7.—Anus. 8.—Ventral Vulva. 9.—Urachus. 10.—Right Umbilical A. 11.—Ventral Bladder. 12.—Right Ventral Ovary. 13.—Ventral Uterus. 14.—Right Ventral Ureter.

vulva lay immediately above the anus, and here there was a prominent darkly pigmented projection composed of erectile tissue resembling the clitoris. The ventral genitalia (Fig. 3) differed from the normal only in being slightly smaller than one might expect them to be. The dorsal genitalia were very considerably reduced in size and altered in form. The uterine cornua, which lay ventral to the smaller kidneys, united after a short course to form a narrow tube about

eight inches in length. The posterior half of the tube, representing the vagina, became closely applied to the elongated bladder and the two passed backwards dorsally to the rectum to open above the anus at the diminutive vulva.

The general arrangement of the skeleton will be apparent from Fig. 4. The left and right ribs of D and S respectively met at one sternum, whilst the right and left ribs of S and D respectively joined the other. The vertebral columns were quite distinct except in the sacral region, where the left lateral edge of the sacrum of D was united to the right edge of that of S. Each half of this compound sacrum, however, had a complete neural canal with the usual superior and inferior sacral foramina. A peculiarity was noticed in the cervical vertebræ. The second bone of S and the fourth bone of D carried, on their left and right sides respectively, additional processes and a complete intervertebral foramen. This additional portion of each of these bones represented what would normally have been the adjacent half of the immediately succeeding vertebra. As a result

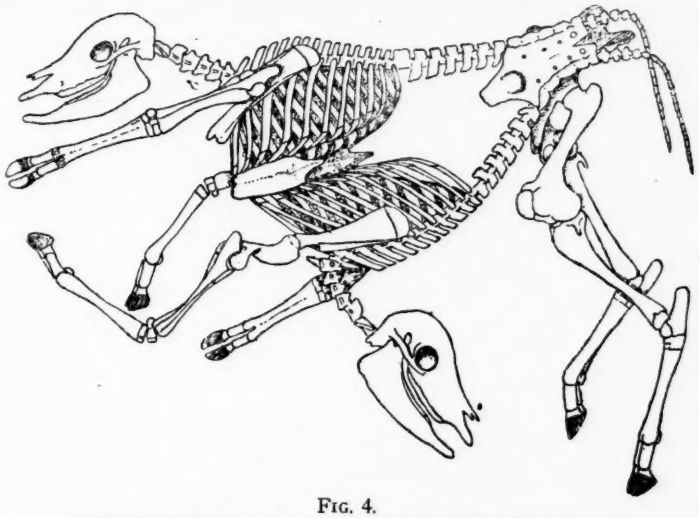

FIG. 4.
SKELETON OF CALF VIEWED FROM LEFT SIDE.
Note that the first three ribs on the left side of D are concealed by the fourth rib.

of these irregularities, all the succeeding vertebræ appeared asymmetrical, the abaxial half of each vertebra, i.e., the half on the left side of S and on the right side of D, corresponding to the axial half of the vertebra immediately succeeding. This asymmetry was particularly noticeable in the sacral region, for here each half of the compound bone showed anteriorly on its axial aspect a transverse process similar to those of the lumbar series, whilst opposite this, on its abaxial aspect, there was a wing bearing an auricular facet for the ilium. A median groove, bounded on either side by the bodies of the sacral vertebræ and above by the opposed lateral portions of these bones, accommodated the dorsal pelvic viscera. A fourteenth rib was present on the left side of D, and this side of the last dorsal vertebra was accordingly made up of two elements. The first right rib of S was arrested in its development and consisted only of a short upper extremity. The ninth and tenth dorsal vertebræ of S (counting on the right side)

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were fused on the right side, but a slender rib projected from the fused mass about half-way across. The innominate bones, which articulated with the auricular facets of the sacrum, met each other below at a normal symphysis. Each carried a cotyloid cavity for articulation with the head of the femur of the normally developed hind limb. In front of the conjoined sacrum was a short, irregular plate of bone which was the only vestige of the skeleton of the undeveloped hind limbs. It received the insertions of the muscles of the ventral wall of the left compartment of the abdominal cavity.

Discussion

From the description given above, it is evident that the extent of duplication occurring in the early embryo is considerably greater than one would suppose from a casual examination of the exterior of the specimen. The embryonic medullary tube, for example, must have been duplicated throughout its extent, for the two spinal cords were quite distinct and spinal nerves were present on both sides of each individual, even in the conjoined sacral regions. It would appear that the sclerotomes had been involved in the fission, even in the sacral region. That their development generally had been disturbed is evident from the asymmetry occurring in each of the vertebral columns. During development, it might be assumed, the central axes of the two embryos would diverge from one another in front of the sacral regions, so that the right side of D and the left side of S would become concave, with a resulting compression of the tissues on these sides. This compression might cause in S the anlagen of the left sides of the two sclerotomes behind the atlas to unite with the anlage of the sclerotome of the right side of the axis. And similarly in D, the elements which would normally become the right sides of the fourth and fifth cervical vertebræ might coalesce with the anlage of the left side of the fourth cervical vertebra. The composite vertebra in S, which represented the ninth and tenth dorsal vertebræ (counted on the right side of the individual), might also have resulted from a coalescence of the anlagen of the sclerotomes concerned on this side, although in this case the coalescence would not be so complete as it was in the cervical region. The presence of a fourteenth rib on the left side of D with duplication of the associated vertebra on this side of the individual might again be accounted for as being due to the divergence of the two embryos, but here, on the convex side of the curve, the pressure was relaxed and a fission occurred.

The primordium of the anterior portion of the gut was obviously involved in the duplication. Evidence of this appears in the two stomachs, the two lengths of intestine(presumably as far as a point a little anterior to the connection with the yolk stalk), the duplication of the spleen, pancreas and the whole of the respiratory tract. The liver, whilst appearing superficially to be a single mass, showed obvious signs of having developed from two distinct anlagen.

With regard to the urino-genital system, it is to be noted that the more normally developed organs, viz., those described as lying ventrally, were derivatives of the abaxial halves of the two individuals, i.e., the right side of D and the left side of S. The duplication of these organs indicates that the ventral portion of the embryonic cloaca was involved in the splitting process, but the dorsal portion must have been unaffected, for the rectum was single.

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A CASE OF DUPLICITAS IN A CALF

Subject viewed from above and on the right.

Subject viewed from below and on the left.

INSEMINATION WITH GELATINISED SPERM IN PARAFFINED CELLOPHANE TUBES

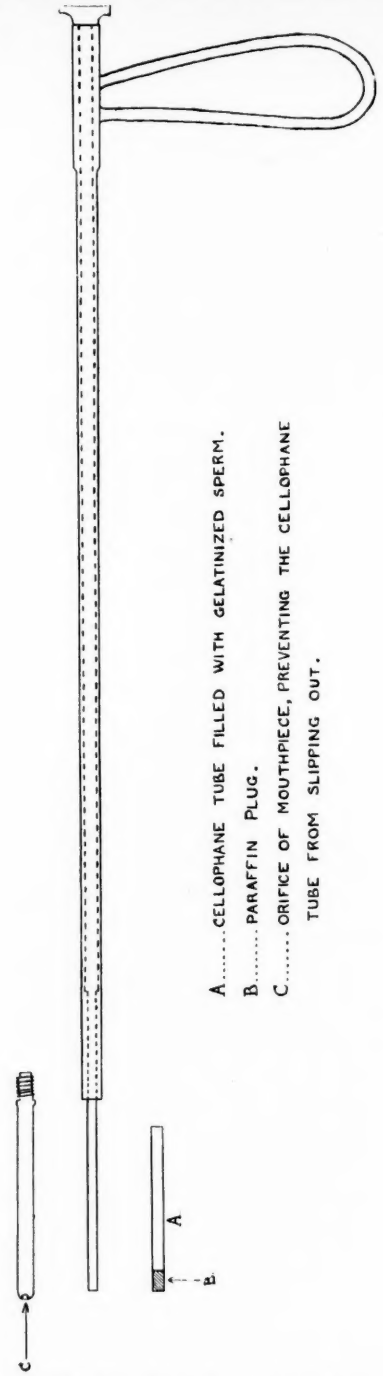

FIG. 1.—Inseminator. Instrument for transfer of gelatinised sperm from paraffined cellophane tube. (For further explanation, see the text.)

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The presence of the two dorsal aortæ, associated as it is with the duplication of the liver, leads one to assume that the primordia of two separate hearts were originally present, but that the development of the heart of S was arrested. The heart of the stronger twin (D) presumably prevailed over that of the weaker, and drove blood through the anastomosis of the umbilical arteries along the aorta of S in a direction the reverse of the normal. The changes in the arteries in the region of the heart of S can be explained by assuming that the development of the arteries of the first three branchial arches was normal, but that in time blood passed down from the dorsal aorta *via* the arteries of the fourth arches to the ventral aorta, and thence backwards to the arteries of the sixth arches (the pulmonary arteries). The peculiar vein which connected the innominate and pulmonary veins of S with the right auricle of D would appear to be formed as a result of a persistence of an anastomosis between the anterior portion of the umbilical vein of S with the sinus venosus of S on the one hand, and the left posterior cardinal vein of D on the other.

The writer is indebted to Mr. J. W. Dransfield, M.V.Sc., M.R.C.V.S., for the illustrations represented in Figs. 1 to 4.

INSEMINATION WITH GELATINISED SPERM IN PARAFFINED CELLOPHANE TUBES

By EDUARD SORENSEN

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In 1938 Milovanov reported that in Russia attempts were made to inseminate animals with sperm suspended in solidified gelatin. The method had been employed for insemination of sheep, with satisfactory results. Experimentally, the method has been used also for insemination of cows, but so far no information has been given about the results.

In 1939 I tried out Milovanov's method, the principle of which is as follows: The sperm is diluted with a gelatin diluent and transferred into small gelatin capsules (capsulæ operculatæ). A capsule is introduced into the cervical canal, where it soon melts at the body temperature and the gelatinised sperm becoming fluid at the same time. From the cervical canal the spermatozoa will then travel to the site of fertilisation, in the uterine tube.

It proved difficult, however, to carry out this method in practice, for it was difficult to obtain gelatin capsules of a suitable diameter and the introduction of the capsules into the cervical canal was troublesome; in heifers, with a narrow cervical canal, it proved practically impossible. As, however, the use of gelatinised sperm offers several advantages—e.g., accurate dosage, prolongation of the lifetime of the sperm cells *in vitro*, and only a slight risk of transmission of infection—I have worked out another method, viz., insemination with gelatinised sperm in

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paraffined cellophane tubes. This method has provel easy and reliable, and since 1940 it has been employed by the great majority of the veterinary surgeons in Denmark who carry out artificial insemination.

The cellophane tubes used for the sperm transmission are the ordinary tubes (" straws ") used for ice-cream soda and other beverages, about 20 cm. long and 3 mm. wide. They are inexpensive and fairly strong, and may be divided with a pair of scissors without the lumen being deformed. In order to ensure that the tubes cause no injury to the sperm cells, they have to be paraffined. This is easily done as follows : With a pair of fine forceps a tiny plug of cotton is inserted into one end of the tube and melted paraffin (melting point 56-58° C.) is sucked up in the tube to the cotton plug. The paraffin is then blown out again, and the inside of the tube is left coated with a thin layer of paraffin that does not affect the sperm cells and greatly facilitates the expulsion of the gelatinised sperm. When the paraffin is blown out of the tube, as a rule, a drop of paraffin will remain in the end of the tube, sealing it effectively, so that it will not be contaminated on storage.

For dilution of the sperm I use the phosphate-glucose-gelatin mixture described by Milovanov and composed as follows :

Distilled water	100 c.c.
Na_2HPO_4, 12 H_2O	1.7 g.
KH_2PO_4	0.07 g.
Na_2SO_4 (anhydrous)	0.08 g.
Glucose	2.85 g.
Gelatin	5.0 g.

This diluent is solid at ordinary room temperature, and becomes fluid at body temperature.

The cellophane tubes are filled and sealed as follows : The gelatinous diluent is placed in a water-bath at 35 deg. C., at which temperature the gelatin soon liquifies. Then the sperm is diluted with the diluent; the degree of dilution is adjusted according to the fertility of the sperm. Following the observations of Phillips and Lardy that egg yolk is a good preservative for bull sperm *in vitro,* it is usual in Denmark to mix the gelatin diluent with 10 per cent. fresh egg yolk. While the diluted sperm is still fluid it is sucked up into the cellophane tubes, which then are placed on a clean piece of paper or in a special rack. The gelatinised sperm solidifies rapidly and the filled tubes are then sealed with a plug of paraffin. This sealing of the tubes is very important and has to be carried out carefully, as the paraffin plug serves not only to close the tube but, later, acts as a piston in the evacuation of the sperm from the tube. As the cellophane tubes vary a little in diameter, it would not be practicable with a metal piston of a given diameter to empty all the tubes completely of the sperm, as some of the sperm would slip up past the piston. After many experiments with various means of sealing, I hit upon the simple paraffin plug, which, when fitting well, is perfectly reliable in causing complete evacuation of the gelatinised sperm. The paraffin plug has the advantage that it makes no difference if the tubes vary a little in diameter. This plug is formed by dipping the ends of the tube into paraffin that has commenced solidifying, having thus a temperature of about 30 deg. With the tip of the finger a little of the soft paraffin is forced into the tube, which then is ready for use and fit for transport at optimal storage temperature.

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For evacuation of the gelatinised sperm from the cellophane tube I have constructed the instrument shown (page 235), called an inseminator. It consists of a metal tube measuring 40 cm. in length and 8 mm. in diameter, provided at its posterior end with a handle, at its anterior end with a screw thread for a mouthpiece measuring 7 cm. in length and 5 mm. in diameter. The mouthpiece, which is wide enough to accommodate the cellophane tube, is bored out so that its anterior end forms an orifice with a diameter smaller than that of the lumen of the mouthpiece, thus preventing the cellophane tube from slipping out of the mouthpiece when the sperm is forced out of the tube. A metal rod passes through the entire length of the tube and mouthpiece. The diameter of this rod is adjusted precisely to the lumen of the tube, so that it will not slip out. The anterior part of the rod, a little longer than the mouthpiece, has a diameter corresponding to the lumen of the cellophane tube.

The inseminator is used as follows: The mouthpiece is unscrewed and the rod is pulled back so that its anterior end projects a little from the tube. A cellophane tube containing gelatinised semen is divided at the middle, and one half is mounted on the anterior end of the rod by forcing the paraffin plug a little way into the tube. The rod with the mounted cellophane tube is pulled back and the mouthpiece is screwed on. Then the rod is cautiously pushed in until the cellophane tube rests against the orifice of the mouthpiece. Now, by means of an even pressure, the gelatinised sperm can readily be forced out of the cellophane tube.

Another way of evacuating the sperm is to place the cellophane tube directly in the mouthpiece before this is screwed on to the tube. The rod is then pushed in till it touches the paraffin plug. For this procedure, however, it is necessary to make the cellophane tube a little shorter than the mouthpiece. With the former procedure, of course, one is quite sure of the proper contact between the metal rod and the paraffin plug serving as piston.

CLINICAL GAS-GANGRENE IN THE NECK OF A BLACK GREYHOUND TREATED BY OXYGEN INFILTRATION

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and J. A. BALDWIN, M.A.

Subject: A black greyhound bitch—Molly. *Age*: 7 months. *Weight*: 36 lb.

History.

Sequence of events was as follows:—

1944. Tuesday, May 16. A telephone call was received stating that this bitch had had a severe injury to the lower left lateral aspect of the base of the tongue and involving the buccal mucosa. A circular wound, ½ inch in diameter, penetrating internally to an area just above and to the left of the hyoid, had been

caused by a piece of rusty steel, 2 feet in length over all, with a hooked end for pegging up hop poles.

At 4.15 p.m. the puppy had been found by the kennel maid with the hook wedged in its mouth and two or three other puppies pulling on the protruding end. With great difficulty and the help of two farm hands, she managed to get the hook out of the puppy's mouth. This caused considerable hæmorrhage at the time and the kennel maid telephoned for assistance.

Temporary first-aid was rendered by washing out the mouth with 10 vol. hydrogen peroxide followed by hourly swabbing with T.C.P. (This latter is said to be a 1 per cent. aqueous soln. of trichlorophenylmethyliodosalicyl with an R.W. coeff. of 10 for the pure salt.)

On instructions one tablet (7½ grs. = ½ gram) of M. and B. 693 was given shortly afterwards, and to be continued at six-hourly intervals together with milk and glucose per os as often as the puppy would take it.

The kennels telephoned again at 8.30 p.m. to say that the puppy was salivating profusely with large swellings on either side of the face and throat.

It was impossible to get down that night as this case was 60 miles away. Therefore advised continuing with hourly swabbing of the mouth and wound with T.C.P. and to give the M. and B. 693 (½ gram) at four-hourly intervals. They stated that her respirations were very accelerated and that she was holding her head up and seemed to be having difficulty with breathing.

Wednesday, May 17. A report was received the following morning that the bitch was in a very distressed condition and could only swallow fluids with great difficulty. The swelling had increased and appeared to be extending from throat to sternum. Advice was given to continue treatment as before until arrival.

The greyhound was seen at 2.45 that afternoon. By this time 3 grams of M. and B. 693 had been given by mouth. She was then slightly comatose with a temperature of 106.5° F., pulse 170, dilated pupils, foul smelling hæmorrhagic discharge from mouth, cyanosed tongue and mucous membrane, accelerated respirations and in obvious pain, being very tender to touch. There was a large œdematous swelling involving both cheeks and extending from the posterior aspect of the mandible to the sternum. On palpating this area, it felt spongy and with crepitations. A probe was put through the wound at the base of the tongue in order to ascertain the extent of the injury. It was washed out with a weak solution of Dettol and filled with about 1½ drachms of M. and B. 693 powder. During this syringing a lot of blood clot came out which seemed to give the bitch relief.

Then 6,000 units of antitetanic serum were injected subcutaneously. The bitch was wrapped in a blanket with a hot-water bottle and brought back to the surgery, arriving at about 8 p.m.

She was immediately put on the operating table, and an incision, 2 inches in length, was made over the hyoid with little pain. A piece of tape was soaked in strong Dettol and used as a Seton drainage tube. A very considerable amount of fœtid fluid was drained away.

Half an hour later the temperature dropped slightly to 105.6° F. Then 1 gram of M. and B. 693 was given per os, also 3 oz. of milk and glucose.

XUM

PLATE I.

Top: The hook with blood-stained mud on the tip (right). Puppies pulled on the curved end (left). *Centre:* 12″ ruler. *Bottom:* The needle and tube used for infiltration.

The final result at the conclusion of the infiltrations.

General view of wound. Arrow points to hole through which hook came into neck.

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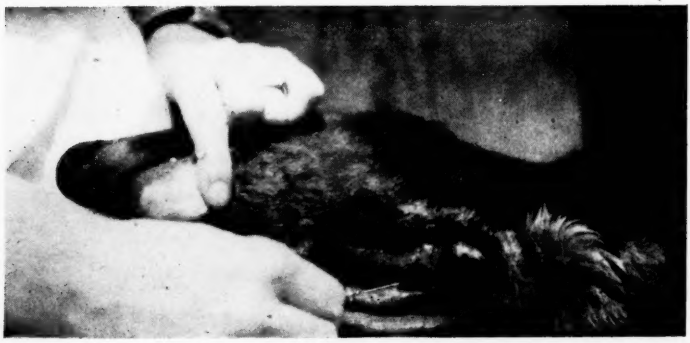

Careful infiltration of thin pretracheal muscle. The deep survical fascia has all sloughed away so exposing muscles beneath.

Friday, June 16*th,* 1944.
By this time wound had almost completely healed without suture. (Right) Hook and foot rule. Initial weight of 36 lb. had increased to 40 lb.

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Respirations were still rapid. Pulse 160. At 11.30 that night temperature was 103.8° F., and the bitch seemed much more comfortable. There was marked improvement in her respirations and a corresponding improvement in the cyanotic condition of the buccal mucosa. Free drainage of the offensive dark coloured brownish matter continued. The pulse remained very rapid.

Later the wound was again syringed out with a solution of Dettol (1 drachm to a pint of warm water) and the bitch again took 2 oz. of milk and glucose. Swallowing seemed to have improved although swelling and œdema around the throat and neck had increased slightly and had extended past the sternum almost to the umbilicus.

Thursday, May 18. At 8.30 a.m. the bitch was in great distress and her temperature was up once more to 106.2° F. The greyhound was examined shortly after and then it was very obvious that (clinical) gas-gangrene was well advanced in all the tissues of the throat and neck. She was again very cyanosed and almost comatose. Gas crepitations could be felt over a wide area. Pulse very rapid and thready; respirations greatly increased.

She was immediately put on the operating table and as there was now apparently no sensation whatever in this area, the original incision was extended for a further 4 inches (total 6 inches long). Reflected back anterior muscles to either side. Further incised fascia and muscles longitudinally. The whole area was gangrenous and most foul smelling. The smell pervaded everywhere and was rather like that of rotten eggs, suggesting, amongst other things, the presence of sulphuretted hydrogen. As much as possible of the underlying necrotic fascia was removed, a great deal of which had turned a greenish colour and become quite soft. But little muscle was taken away apart from friable blackening tissue from the muscle margins which could be easily removed or swabbed off. The original wound from the mouth through into the neck was also opened up to allow more free drainage and the whole area was cleaned with Dettol and warm water.

The reflected sternothyroideus and sternocephalicus muscles were turning black on their margins and had a greenish tinge looking towards the body of the muscle and more distally a dull red. On palpation, drops of greenish brown, serous, fœtid fluid appeared from them. Gas crepitations could be felt over a considerable area around the wound. During all the above operations there had never been any sign of sensation—in this respect the area was as if dead.

The deep cervical fascia was intact above, but showed an ominous trace of a greenish tinge.

In view of the vital areas that were about to be involved and the fact that all previous cases, even where a true radical excision of infected tissue was possible, had died, the situation was definitely becoming distinctly serious.

The wound was left entirely open. On returning from the operating room, the bitch's eyes and expression were dull and listless. The temperature was still over 106° F. Respirations 130. Apex rate 176.

Now there had appeared quite recently in *The British Medical Journal* (April 1, 1944, p. 470) a letter from Dr. N. M. Macdonald describing the infiltration of oxygen for gas-gangrene. It was therefore decided to try out this method. (It is also mentioned in Handfield Jones and Porritt's "Essentials of Modern Surgery," p. 147.)

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Shortly after 12 noon the greyhound was returned to the operating table and the wound uncovered. A rubber tube was connected by one end to an oxygen cylinder of the anæsthetic apparatus and by the other to a holder to which was fitted a No. 2 hypodermic needle—this was kept sterile in a dish of methylated spirit. The oxygen was turned on and the flow of gas was adjusted to a moderate rate by observing the bubbles issuing from the needle under the spirit. The rubber tube was not attached too tightly to the oxygen cylinder valve, since if the oxygen was turned on too much or the needle became blocked, the tube was blown off so acting as a safety valve. The needle was then inserted into the visible muscle areas and oxygen allowed to spread slowly up and down the tissues, also into the subcutaneous margins of the wound and into the deeper muscles of the neck and throat, care being taken to avoid veins, etc., and to keep the oxygen pressure low especially when near such structures owing to the danger of causing an air embolus. This was continued until oxygen emphysema could be felt well down the neck below the wound, and well above it. There was no evidence of pain or of contractility in the muscles. The reflected sternocephalicus looked dry on the surface and the blackening margins were friable. Her mouth was also opened and oxygen injected deep below the cyanosed mucosa—so blowing up the whole cheek on each side with oxygen. Within a very short time this mucosa assumed an almost normal pink colour. By an oversight the needle was not changed for the sterile needle lying in the dish, but no harm resulted.

In the wound itself the local changes to the muscle tissue were dramatic, colour altering from a dull red and greenish colour to the bright red of oxyhæmoglobin. Even parts of the black margins, where oxygen was blown in extra thoroughly, showed some recovery. (Small pieces of these blackened friable margins were the only parts of muscle removed at any time.) Greenish infected fascia showed up still more by contrast, since there was no improvement in this tissue by the oxygen treatment. The deep cervical sheet of fascia was still intact above but had a dead white colour with here and there a greenish tinge. It was decided to leave this intact and await developments. The state of the thin muscles beneath, over the thyroid cartilage and trachea, was not known, but if they had received any oxygen, it was by indirect saturation, since it was decided not to push the needle blindly through this fascia. Finally the oxygen was turned to a higher pressure and blown all over the surface of the wound and especially into the parts that had been undercut beneath the skin below the jaw, and into the small penetrating wound from the mouth.

On returning from the operating room, the bitch appeared distinctly better, though hardly cheerful as her cheeks were considerably blown up with oxygen. It was decided to use the cheeks as a guide to when absorption of oxygen was completed.

Improvement was marked and two tablets of M. and B. 693 were given morning and evening. Total M. and B. to this morning, therefore, was 5 grams.

Further, it was decided, in order to keep the conditions of the experiment as simple as possible, to withhold the gas-gangrene antitoxin. The one unfortunate feature was that, for various reasons, it was not possible to get a culture done from the wound.

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By about 6 p.m. the swelling of the cheeks due to the oxygen had completely subsided, and they appeared perfectly healthy, so that it was decided that only the wound itself should be infiltrated in future. The bitch was definitely more normal and no longer bordering on being comatose as she had been in the morning. The complete apathy had gone. She also took some more milk and glucose. Her respiration rate, though still up, had fallen, her apex rate was 170, and her temperature was 104.4° F.

That evening at 10 p.m. the wound was again examined and the muscles still showed their marked improvement, though the black margins at the free edges had returned. These margins were reinfiltrated especially carefully, but no doubt retained the oxygen less easily than the body of the muscles. The deep cervical fascia had now turned more green and had broken away in the centre exposing the thin muscle layer over the trachea beneath. The oxygen treatment appeared to be having no effect on this fascia, possibly because it could not penetrate such tissue satisfactorily. What was visible of these muscles was lightly infiltrated by holding the needle parallel to the surface of the muscle, the point just beneath, and keeping it there for some while with a very small flow of oxygen. Some pieces of necrotic fascia which easily came away were removed with forceps. There was still little sensation anywhere. Oxygen was also blown into the wound from the mouth and over the whole wound surface. The wound was then recovered with a dressing, no local antiseptic being used, and the bitch was returned to her bed. The local visual improvement of the muscles as the oxygen flowed in was again dramatic. (The myohæmoglobin and cytochrome of muscle must greatly facilitate the retention of oxygen by this tissue.)

A great deal of the improvement of the central part of the muscles had remained since the morning, though not so much in the margins. Fascia continued to turn green and slough away, however. There was now little or no smell from the wound.

The general improvement was maintained most satisfactorily. At no time had there been any vomiting. Two tablets of M. and B. 693 were then given per os. Total now—6 grams by mouth and 1½ drachms into the wound, to this evening.

Friday, May 19. Morning. Respirations remained improved. Temperature 103.2° F. Apex rate 122. About 12 noon, oxygen infiltration was carried out again as described. The muscle had remained a healthy bright red colour which was further increased by the fresh re-infiltration. But the sheet of deep cervical fascia was completely green and breaking up with here and there patches of white sloughs. Now the thin pretracheal muscles underneath could be infiltrated carefully with oxygen. They remained healthy and uninvaded in spite of the fascia sloughing over them. Much of this was pulled off with forceps, leaving only a few small pieces, that were still too much attached to the muscles below, to come away later. There was some very definite return of sensation and a slight degree of obvious pain was caused by this infiltration. The black patches on the margins of the sternocephalicus and sternothyrohyoideus were now almost gone. These (apart from the small necrotic pieces that had been taken away at the initial operation) had not been removed, but had been given extra long treatment under

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higher pressure. This time the wound was covered with a thick 1 :1,000 water-in-oil acriflavine emulsion dressing.

General condition showed continued remarkable improvement. During the day the bitch ate some meat and drank water voluntarily and was much brighter. In the afternoon she was able to walk in the garden quite normally.

Evening temperature was 101.2° F., respirations were about normal, and apex rate was 130. At 7 p.m. further oxygen treatment was carried out—much more sensation being present, and at times the bitch tried to draw her head away from the needle. The final tiny sloughs of the deep cervical fascia that remained were picked away or cleaned off with a swab. The whole wound and all the muscles now looked very healthy and a bright red colour throughout. When finally blowing oxygen into the cavity of the wound from the mouth a vessel was pricked and caused some bleeding of bright red blood. This was stopped satisfactorily and gave no further trouble. A fresh acriflavine dressing was applied to the wound and the bitch returned to her bed. ½ gram of M. and B. 693 was given to-day, morning and evening. Total M. and B. 693 given was now :—By mouth, 7 grams; into the wound, 1½ drachms.

Saturday, May 20. 12 noon. Temperature 101.1° F. Apex rate 130. Respirations normal. No M. and B. 693 was given. Apart from the large open wound in the neck and the neck dressing, the greyhound was almost normal in behaviour, allowing for the fact that she was of a very timid and nervous disposition.

At 12.30 p.m. the wound was uncovered and oxygen was blown all over its surface, and particularly into the undercut parts. The photographs of it were taken at this time. Only slight direct infiltration into the exposed muscles was carried out, as the whole appearance was normal. It was chiefly as a prophylactic against any recurrence. During the day she was eating and drinking well and walking very satisfactorily. The threat that had arisen from the gas-gangrene was now considered to be definitely at an end.

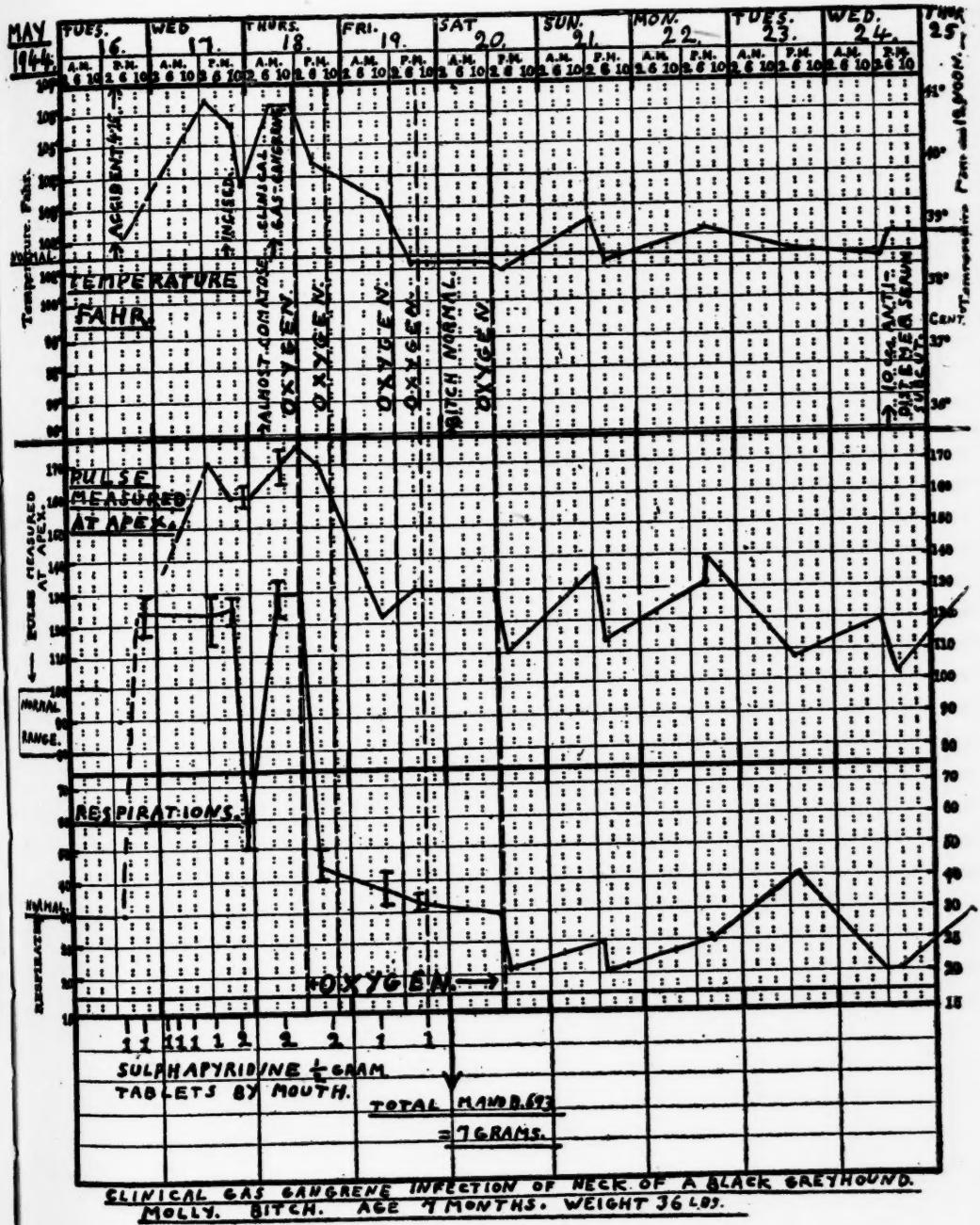
4.30 p.m. Respirations 21. Temperature 101.0° F. Apex rate 110. The acriflavine dressing was changed, but no further oxygen infiltration was carried out. No further M. and B. 693 was given.

At the times the infiltration was in progress there was some cardiac acceleration, partly due to nervousness at being on the table, but no serious disturbances of rhythm were noticed. Owing to the almost complete loss of local sensation by the time the neck was incised, no anæsthetics or analgesics were necessary.

Summary

The greyhound had been under M. and B. 693 by mouth from soon after the time of wounding, yet this appeared to be having no effect upon the onset of the gas-gangrene, although it may have been holding any coincidental ærobic infection under control. The onset of clinical gas-gangrene appeared to be in spite of its administration. The anærobic infection was perhaps of the milder variety, since the temperature was high, and not subnormal. In this respect it was unfortunate that no culture was possible.

The infiltration of oxygen appeared to have a direct and dramatic effect upon the muscles but none whatever on the badly infected fascia, though it may have



CLINICAL GAS GANGRENE INFECTION OF NECK OF A BLACK GREYHOUND.
MOLLY. BITCH. AGE 9 MONTHS. WEIGHT 36 LBS.

stopped further infection of healthy fascia beyond the wound since no spread occurred along the fascial planes. Probably, unlike muscle tissue, fascia will not take up oxygen sufficiently for any effect. The two following statements in the letter to the *B.M.J.* mentioned, were confirmed, at least in so far as this one case is concerned:—

"The improvement, local and general, was as dramatic as had been the onset and spread of the gangrene," and, "One fact, proved beyond any shadow of doubt to all those connected with this case, was that the gas-gangrene was not only arrested, but cleared from heavily infected tissues by the intramuscular infiltration of oxygen." The second part of this last statement was borne out in regard to muscle tissue, but not in respect of fascia.

It must also be admitted that no culture appears to have been obtained from the case mentioned in the *B.M.J.*, so that neither that or the present example are on a very satisfactory footing. In the case of the greyhound, the antitoxin was deliberately withheld and no local antiseptic was applied after the infiltration of the oxygen until it was obvious that improvement was taking place;—this was to keep the conditions as simple as possible.

Owing to the site of infection being the neck and the fact that this greyhound was intended for racing, radical excision was not feasible except to the very smallest extent.

Finally it must be emphasised that this was not just a single infilration with oxygen, but a continued closely observed process—a maintained attack requiring care and time. Indeed one treatment alone might well have been insufficient.

No exact assessment of the foregoing can really be made until further examples of gas-gangrene in dogs are found and subjected to similar treatment, together with cultures made before and after oxygen infiltration.

Substances Used

Anæsthetics or Analgesics: None necessary, owing to the almost complete loss of sensation by the time neck was incised.

Locally: T.C.P. and Dettol into the wound with apparently slight effect. The 1:1,000 acriflavine emulsion was not used until twenty-four hours after the first oxygen infiltration by which time there had already been a most marked improvement, both locally and generally. (Proflavine could not be obtained in time.)

Antitetanic serum: 6,000 units subcutaneously, twenty-four hours after wounding.

Antitoxin: None given, though it was obtained in case of need.

Surgical excision: Slight, but area opened very widely and drainage excellent.

M. and B. 693: *Into the wound,* about 1½ drachms of powder were packed into this about twenty-four hours after occurrence. Much of it was probably washed away by subsequent irrigation. *By mouth,* from time of wounding over following three days—*7 grams.*

The wound healed completely and uneventfully without any suturing, and by Sunday, June 11, the dog had increased 4 lb. in weight to 40 lb.

In view of the increased interest in this subject in time of war, the writers

would be grateful to learn of any results obtained by others using this or other methods, and of the nature of cultures where these have been possible. By this means some idea of the degree of response of the various causative organisms to these methods in differing species of animals could then be ascertained. While it is true that Clostridium welchii, septique and œdematiens have been found to be sensitive to the action of penicillin, yet, with an infection of at times such virulence, the search for, testing and assessment of simple adjuvant or even alternative measures is likely still to be of considerable value, especially as in the case of animals amputations are so much less feasible a proposition.

Biochemical Memo. (Provisional)

In Notes upon Gas-Gangrene, Medical Research Council War Memorandum No. 2, revised 2nd edition, p. 17, para. f No. 3, it is stated "that Sir Almroth Wright (*Lancet*, 1917, 1, I.) pointed out in the war of 1914-1918 that gas-gangrene produces severe acidosis, but that little work has since been done on this subject."

It was wondered therefore whether the vomiting that sometimes occurs in gas-gangrene might be partly due to this acidosis, and whether the acidosis might arise to some extent from an excessive accumulation of lactic acid in the infected muscle tissue. Under anærobic conditions such lactic acid, once formed, would accumulate, since it would remain unoxidised. (In muscles caused to contract under anærobic conditions, lactic acid accumulates.—T. R. Parsons.)

Further, "the central position that cytochrome occupies in the oxidation mechanism of the cell" (Parsons) might be upset, especially if its oxidising enzyme, cytochrome oxydase, (indophenol oxidase), were to be inactivated, for example by hydrogen sulphide formation in even small concentrations. Such inactivation of the cytochrome oxidase would prevent reduced cytochrome being reoxidised by atmospheric oxygen.

Therefore in the severer forms of gas-gangrene, or in gas-gangrene that has passed a certain stage, it might prove that no amount of infiltration of "atmospheric" oxygen alone could of itself restore the damaged tissues, because the oxidation mechanisms of the cells had been upset too far. However the cytochrome-cytochrome oxidase system might be temporarily replaced by e.g., methylene blue. (The oxygen uptake of the tissues is completely inhibited by small concentrations of H_2S and KCN, and by CO in the dark.—Sydney W. Cole.) The peculiar stench from the neck wound in this greyhound had suggested traces of H_2S being present. Other similarly acting pigments might also be tried.

After the oxygen infiltration most of the muscle had tended to change to a brighter red and remain thus for a long period. Since the hæmoglobin in the blood would soon be carried away in the capillaries and vessels, this change was thought to be due to oxygenation of the myohæmoglobin direct.

Oxygen infiltration does not appear to be mentioned in the Medical Research Council's War Memorandum No. 2, but the growth of strict anærobes in vitro in the presence of air is described, by the addition of small quantities of agar and of reducing substances to previously heated culture media, or by the addition of reduced iron in the form of small strips of steel sheeting, or non-rusty filings or nails, to make up for example iron-milk or iron-gelatin media, such methods affording a sufficient measure of protection to the bacteria to allow vigorous multiplication to take place.

XUM

Kocholaty and Hoogerheide have made a detailed study of the metabolism of Clostridium Sporogenes (*Biochemical Journal* 32, i, 1938, 437 and 949). They conclude that "true" fermentation reactions, Stickland intermolecular fermentation reactions and intermolecular oxido-reductions where one molecule of a substrate substance is oxidised and the other reduced, since many substrates act as H donators as well as acceptors, appear to be the main sources of energy of Cl. Sporogenes. The oxido-reductions are independent of the presence of H_2, and occur also as side reactions accompanying H_2 uptake.

They found that washed suspensions of Clostridium Sporogenes could bring about the reduction of amino-acids and other substances by the active utilization of gaseous hydrogen, especially when glucose was present in the culture medium, though this was not entirely essential. In general it was observed that substrates could be attacked more easily at low pH in the acid ranges, apparently by the formation of adaptive dehydrogenases. The pH optimum for the hydrogenase enzyme was about 7.1. Glucose was the only substrate from which much H_2 was evolved.

Some fermentation reactions were found to take place in an atmosphere of N_2, there being no H_2 uptake and they are independent of the presence of glucose in the culture medium. With regard to the H_2S formation mentioned previously, "when the sulphur containing amino-acids cystëine, cystine and methionine were used, active fermentation occurred with H_2S as the chief gaseous product," and in their case, in an atmosphere of hydrogen, much H_2 is absorbed.

It is possible that the infiltration of free gaseous oxygen O_2 may inhibit or reduce the activity of certain of these reactions and their necessary enzymes but until more cases of gas-gangrene have been treated by the infiltration of oxygen, the organisms concerned isolated and the results assessed it would seem undesirable to comment in detail on these matters.

However it may be pointed out that the authors quoted comment that "the extreme lability of the dehydrogenases of Cl. Sporogenes, even under anærobic conditions at 0°, is remarkable and in striking contrast to that of Bact. Coli, a suspension of which may be kept for a considerable time in the ice-box without loss of activity." The addition of $CuSO_4$ even in concentrations as low as 5×10^{-4}M inhibited completely all dehydrogenation reactions on the three substrates so tested.

They studied the influence of variation in enzyme concentration by noting the effect of decreasing concentrations of washed suspensions of Cl. Sporogenes used for dehydrogenation reactions with the substrate *d*-alanine, other factors being kept constant. They found that "there was by no means a constant ratio between reduction time and bacterial concentration in the entire range of the dilutions. An approximately constant ratio exists only when rather high concentrations of bacteria are employed. The lower the bacterial concentration, the more pronounced the deviation from the hyperbolic function.

"Probably this is due to the fact that during the manipulations in which a limited contact with air is difficult to avoid, a small part of the activity is lost. As a matter of fact, smaller amounts of the suspensions or dilute suspensions are less protected against oxygen and a loss of activity is the consequence." This

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suggests that the earlier the oxygen infiltrations can be commenced, the greater the chances of success, and that preliminary removal of dead tissue and drainage should be as complete as possible.

Similarly when studying the hydrogenase enzyme of this same organism capable of activating H_2 they observe that "it was found necessary to exercise great care in eliminating traces of air from the anærobic culture jars. Traces of air, which with heavy inocula apparently exert no influence on bacterial growth, very markedly diminish the H_2 absorption of bacteria obtained from such a culture. For the same reason it was found necessary to remove O_2 traces from buffer and substrate solutions by boiling them just before use."

Too much emphasis must not be placed upon the reactions found with one species owing to the differences that have been observed from one species to another. Thus "recently Woods and Clifton (1937) have shown that Cl. Tetanomorphum decomposes many amino-acids with the liberation of H_2. Cl. Sporogenes behaves very differently in this respect inasmuch as none of the amino-acids tested but only glucose yielded H_2." This indicates the necessity of obtaining cultures wherever possible and identifying the species concerned when the effects of oxygen infiltrations are being studied.

In the more superficial types of gas-gangrene multiple incisions alone are said to be very effective. This is presumably because culture media such as blood clot, etc., are drained away and infected tissues well exposed to the oxygen of the atmosphere. In more massive gas-gangrene infections where muscle has been extensively involved and particularly where débridement has not been thorough, or, as in this instance, has not been possible, the drainage, and more especially the exposure to molecular oxygen, cannot be anything like so complete or effective. Hence the importance of *continued* infiltrations of all the tissues with gaseous ozygen in these cases. Ischæmic areas of muscle due to vascular damage may conceivably be kept viable, by the direct supply of oxygen to the tissues and by the inhibiting effect upon bacterial growth, until alternative circulations are established providing that these are anatomically possible. The mere inhalation of oxygen in high concentration is unlikely to benefit areas of poor circulation, i.e. just those parts in which bacterial multiplication will be the most rapid, though it might assist in reducing the rate at which they can spread. Infiltration on the other hand reaches directly to these sites.

The oxygen probably does not kill the organisms but inhibits enzymic activity which will recommence when the oxygen has been all absorbed. Such a tendency to recur was observed in the example described. Therefore it is desirable to carry out repeated infiltrations to keep the oxygen in as high a concentration as possible. The severer the infection the more thorough must be the infiltration since it would appear certain that the higher the bacterial concentration the less easily will it be affected by molecular oxygen. For these reasons a single infiltration of oxygen is not likely to have more than a transient effect except with very mild infections due either to a relatively inactive strain or where the quantity of organisms is small. The inhibitory effect of the oxygen upon the organisms will then give the defence mechanisms of the body a greater chance to come into effective action against them. Repeated infiltrations are also advisable to prevent

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the possibility of the organisms recovering and adapting themselves to the changed conditions, perhaps by the formation of resistant spores or by other means. In this respect both Cl. Sporogenes and Cl. Histolyticum were found in the paper mentioned to adapt themselves quickly to more acid conditions of pH in the culture medium, pH 7.4—→pH 6.4, the pH optima for the various dehydrogenation reactions being then shifted to lower pH values and the explanation put forward was that the organisms might be synthesizing slightly different (adaptive) enzymes more suitable to cope with them. They suggest that this phenomenon of adaptability is probably to be found throughout the entire microflora.

(We wish to record our grateful thanks to Dr. C. J. C. Britton, of the Middlesex Hospital, and to Dr. Tom Hare, of the Veterinary Laboratories, London, for their kind help and interest in this work, and to the father of one of us, Mr. Aslett Baldwin, F.R.C.S., who also drew our attention to the possibilities of this method in the first instance.)

THE HORMONAL TESTS FOR EQUINE PREGNANCY

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Introduction

For a number of years workers in various parts of the world have been using hormone tests for the diagnosis of equine pregnancy. A number of different tests can be used, which fall into two groups, viz.: (1) Tests for a high level of gonadotrophic hormones in the blood during the earlier stages of pregnancy; and (2) tests for a high level of œstrogenic hormones in the urine in the later stages. The tests for gonadotrophic hormones in blood are biological and were originally described by Cole and Hart[3]. The tests for œstrogenic hormones in urine are either biological or chemical, the biological method most commonly used being based on the technique originally devised by Zondek[11]. A chemical test was first devised by Cuboni[4] and since his original description various modifications have been suggested[1, 10], but these modifications are all of a minor nature. Kober[5] devised another chemical test for œstrogens and this, as originally described or with modifications[2], has been successfully used to diagnose pregnancy in the mare.

Of the tests mentioned above, the biological test for œstrogens using ovariectomised mice as test animals, the Cuboni chemical test for œstrogens, and the biological test for gonadotrophic hormones using immature female mice have been most widely used in the diagnosis of equine pregnancy. A considerable degree of success using these various tests at the appropriate stage of pregnancy has been claimed by various people. Thus, Miller[7] in 1934 estimated that the biological urine test was 99.5 per cent. accurate, although in 1940 Miller and Day[9] published results obtained with an unspecified number of selected mares suggesting that it was considerably less accurate, showing errors varying from 19.5 per cent.

to 76.4 per cent. during different phases of the normal test period of from 90 days onwards. In the latter communication, Miller and Day[9] also reported an error of between 2.9 per cent. and 16.7 per cent. with the blood test carried out during the usual test period of from 40-90 days after service. Mayer[6] in 1944 assessed the accuracy of certain of the hormonal tests and also found it to be high.

Routine pregnancy diagnosis has been carried out in this Department since 1941, and since the authors took over in January, 1944, a sufficiently large number of samples has been examined to throw some light on the general and relative accuracy of the various hormonal tests. As our results do not agree completely with the above, and as the numbers examined have been fairly large, particularly as regards urine tests, it has been thought worth while to publish our findings.

Methods

The techniques followed in carrying out the biological tests for pregnancy were those described by Miller and Day[8], while for chemical examination we used the original Cuboni test. Urine samples were, so far as possible, not accepted until at least 90 days had elapsed since the last service, while blood samples were examined during the period 45-90 days after service, as it has been shown that outwith these periods the respective tests are of little practical diagnostic value. Efforts have been made to ascertain the efficiency of the tests thus used by asking every veterinary surgeon receiving a report to inform us as to the accuracy of our diagnosis when clinical evidence put the matter beyond any doubt; in about half the cases the information requested has been forthcoming.

Results and Discussion

I. *Biological Tests.*

TABLE I.

Tests carried out during Period January 1, 1944, to April 1, 1946.

	No. of Tests.	*Confirmations Notified.*	*Wrong Results Notified.*	*Confirmed Positives.*	*Wrong Positives.*	*Confirmed Negatives.*	*Wrong Negatives.*
Biological Blood Tests	56	22	Nil	14	Nil	8	Nil
Biological Urine Tests	330	156	5	70	Nil	86	5
Totals ...	386	178	5	84	Nil	94	5

(a) *Biological Blood Tests.* From Table I it will be noticed that no errors have been reported with this test. The results of the 56 tests so far carried out appear to indicate a much higher degree of accuracy than has been claimed by other workers.

(b) *Biological Urine Tests.* From Table I it will be seen that the error expressed in terms of all the biological urine tests regarding which veterinary surgeons' reports were received was about 3 per cent.; however, as there was every likelihood of wrong diagnoses being brought to our attention, it is highly probable that the real error was considerably less than this. An examination of the mistakes in diagnosis showed that in each case a negative result was obtained where the mare subsequently proved to be in foal; in the cases wrongly diagnosed, one mare had been served 90 days before collection of the urine sample, one 100

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days, one 109 days, one 135 days, and one 213 days. The mare which gave a negative test at 135 days gave a definite positive test 90 days later, by which time the veterinary surgeon suspected pregnancy from clinical evidence. If the errors are expressed separately with reference to positive and negative tests, it will be seen that positive results regarding which reports were received were 100 per cent. correct, while the negatives gave an error of just over 5 per cent.; but, as pointed out above, the real error might be less than this.

Various suggestions could be made as to the cause of the errors. It has been suggested by some workers that a small percentage of mares may be later than usual in producing sufficient œstrogen to give a positive result; this is supported by the fact that three out of our five erroneous results were samples from mares not far beyond the usual minimum period of 90 days after service, and one of these animals, when tested later, gave a good positive reaction. Another possible explanation might lie in the fact that on a succulent diet the urine is usually much greater in volume than on a dry diet, and the consequent dilution of œstrogens would tend to upset a quantitative test; it is possible, therefore, that errors might be reduced by having the mares on a dry type of diet at the time the urine sample is taken. It is especially interesting that the type of error we encountered with this test did not agree with Mayer's findings[6] that out of 97 tests carried out, four mares giving positive biological urine tests did not produce foals. We have never encountered an error in this direction. It is also interesting that our percentage accuracy using this test was much higher than Miller and Day[9] found in 1940.

II. *Cuboni Chemical Test.*

TABLE II.

Comparison of Results using Cuboni Chemical and Biological Urine Tests simultaneously.

1	2	3	4	5	6
Both Tests+	Both Tests—	Cuboni+ Biological—	Cuboni— Biological+	Cuboni doubtful Biological+	Cuboni doubtful Biological—
126	181	Nil	11	2	6

Between January, 1944, and March, 1946, 326 of the samples of urine submitted to the biological test were simultaneously tested by the Cuboni chemical method—the comparative results are set out in Table II. It will be seen that there was agreement between the two methods in 307 out of the 326 tests carried out, i.e., about 94 per cent.

In all cases where an error in diagnosis was encountered with the biological test, the Cuboni test was also wrong; also, in all cases where the two tests differed, and a report was later received, the biological test was always proved correct. As indicated in column 3, in no case where a positive Cuboni reaction was obtained was the biological test negative, but in 11, or about 3 per cent., of the total results a negative Cuboni test was obtained when the biological test was positive (column 4). It has been indicated earlier that as far as can be ascertained a positive biological test has never been a wrong diagnosis, consequently the error of the Cuboni test (excluding doubtful reactions) would appear to be about 3 per cent. higher than that of the biological test, and the error was again always in

the same direction, i.e., a negative chemical reaction where the mare subsequently proved to be in foal. Presumably, this is due to the Cuboni test being less sensitive to "border-line" levels of œstrogen than is the biological method. If the positive and negative Cuboni tests are considered separately, it will be seen that, when positive, the Cuboni test was apparently 100 per cent. correct, and when negative it had an error of about 6 per cent. greater than the negative biological urine tests, since of the 192 Cuboni tests which were negative only 181 coincided with the results of the biological tests; thus, the accuracy of negative Cuboni reactions may not be greater than 90 per cent. Two of the negative Cuboni tests which were in disagreement with the biological test, though read as negatives immediately after completing the test, were positive when the tubes were re-examined some 24 hours later; the same experience was encountered in the case of one doubtful test (column 5). It would, therefore, appear that the error of the Cuboni test can be reduced slightly by re-reading the test 24 hours after completion. Eight of the Cuboni tests which did not agree with the biological tests were at a stage earlier than 133 days from the last service (no service date was available in the other three cases), so that more than 4½ months after the last service the definite Cuboni results appear to have been in complete agreement with the biological test.

Columns 5 and 6 show that in eight cases the Cuboni test could neither be read as definitely positive or negative, but gave a faint green fluorescence which was classified as doubtful. It will be noticed that two of the samples giving doubtful Cuboni tests were positive biologically, while six were negative. Of the two tests where the biological test was positive and the Cuboni doubtful, one was positive 24 hours after completion, and the other was a sample which, owing to faulty history, was examined abnormally early, viz., 84 days after service. In no case has it been necessary to classify a biological test as doubtful.

These results of the comparison of the Cuboni and biological urine tests indicate that for the earlier stages of the test period the biological test is more reliable than the chemical test. The occasional doubtful Cuboni reaction is a further disadvantage, but a positive Cuboni test can, apparently, be accepted as definite evidence of pregnancy.

III. *Fertility of certain types of equines.*

The examinations we have carried out emphasise the very low fertility in equines of the racehorse and hunter types, as the great bulk of the samples examined have come from these types of horses. Of 386 samples examined for pregnancy during the past two years, 167 have proved positive and 219 negative, so that the fertility rate of the sample of animals dealt with would appear to be a little more than 43 per cent.

Summary

Routine use of hormonal tests as a method of diagnosing equine pregnancy during the past two years has thrown further light on the efficiency of these methods. The biological urine test was found to have an accuracy of at least 97 per cent., the errors being confined to negative results and tending to occur mainly in the early stages of the test period; no errors were encountered with the biological blood test.

A comparison was made of the Cuboni chemical test and the biological urine

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test. It was found that the biological test was somewhat more efficient, particularly during the early stages of the examination period, although positive Cuboni reactions appeared to be 100 per cent. correct.

The results obtained in this work emphasise the very low fertility rate in certain types of equines.

Acknowledgments

We are indebted to Miss J. Smith and Miss B. Milne for assistance with some of the routine work.

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A COMPARING-CHAMBER FOR MICROSCOPIC EXAMINATION OF UNDILUTED BULL SEMEN

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In the examination of the fertility of a male animal, microscopic examination of the undiluted ejaculate is a diagnostic method of the greatest importance if it is carried out carefully, *immediately* after the collection of the semen in the artificial vagina. Whether the object is to estimate the fertility of an unknown male or to decide whether a given ejaculate is suitable for dilution and employment in artificial insemination, the main purpose of the examination will be in the quickest and simplest way to get an idea about the *physiological quality of the semen, i.e.,* the amount of fully motile normal sperms per unit volume of the specimen of the semen concerned.

As to the highly concentrated semen of the bull, it is a difficult task to estimate objectively the concentration by ordinary microscopy, as well as to judge of the motility, *i.e.,* the percentage of living sperms* in the specimen. This, perhaps, is illustrated most strikingly by the fact that so far almost every investigator working systematically on this problem has had his own system of gradation. Thus several authors have directly given the percentage of living sperms in the specimens—something I should consider a rather hopeless task if it were to be carried out on an ordinary fresh preparation with merely a fair degree of accuracy. As to more exact microscopic methods, the sperm-count may

*In this paper, the term "living sperms" means invariably sperms showing distinct movements of their own.

be performed on diluted semen in counting-chambers such as are used for blood-cell counts.

As an example of such microscopic estimation of the quality of bull semen by means of a hæmocytometer, it will be appropriate to give the technique that has been employed in this laboratory. A little of the newly collected semen is mixed in a test-tube with a gelatine diluent at body temperature (GPC-3-G, Milovanov, 1938) in the proportion 1:100. After careful mixing, a counting-chamber—Thomas-Zeiss' older, closed model—is filled, and now, at body temperature, all the non-motile sperms are counted in 5 or 10 large squares. Then the counting-chamber with its contents is heated to 60° C. for 1-2 minutes in an incubator to kill all the sperms. A new count gives the total number of sperms per unit volume, and then the number of living sperms per unit volume may be calculated as the difference between the two counts.

Such a technique, in which subjective judgment is practically eliminated, will generally be serviceable only under good laboratory conditions, as it is too involved and time-consuming for ordinary field work.

Author's Investigations

For the gradation of the motility in bull semen, in 1942[1] I suggested a system that is easy to employ in practice, being based on the characteristic mass-currents or wave movements, known to most examiners performing microscopy of normal bull ejaculates at body temperature. In a dense active ejaculate the currents may not infrequently be just macroscopically visible. Immediately after the collection of the semen, and in suitable light, the currents may be seen through the wall of the collecting tube as tiny waves of a mother-of-pearl character. Any attempt at closer study by means of a drop of the specimen on a slide under a cover-slip will usually prove a failure, the waves disappearing completely in such a preparation. If, on the other hand, the cover-slip is omitted, so that there is a thicker layer of semen under the microscope, it is quite practicable, with low magnification (20-50 ×) and proper illumination, to pick out the waves clearly (Fig. 1).

Wave formation is a characteristic feature of motility of normal undiluted high quality bull semen, and is brought about by the mass movement of the closely-packed sperms. According to the rate of motion of the waves at body temperature, distinction has been made between various degrees of motility, designated by means of the following symbols: +++ = rapid "whirls"; ++ = "sluggish waves"; + = "single propulsions"—no mass activity; 0 = necrospermia. In a slightly extended form, this system of gradation will be mentioned further when dealing with the employment of the comparing-chamber.

A good idea as to how this wave motion is brought about may readily be gained by examination of a layer of semen, 100-200 μ in thickness. The fully motile sperms will be seen to "sweep" the slightly motile and dead sperms together into denser and, therefore, darker groups, or, more frequently, streaks, which are made to undulate by the constantly changing forces exerted on them. As the head of the bull sperm is 4-5 μ wide and about 1 μ thick, it is obvious that the inner part of the waves, where the particularly numerous sperm heads in the dense crowd are standing more or less "on edge," may become quite opaque

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on account of the strong reflection (see Fig. 2). The wave motion, therefore, may be interpreted as a " sweeping phenomenon," and differences in the density, width and rapidity of the waves in various ejaculates will be due chiefly to variations in the ratio between living and dead sperms in the specimens.

In a previous paper[2] a detailed account was given of the conformity found between gradation on the above-mentioned " wave principle " and the more exact determination of the motility carried out by the hæmocytometer method mentioned in the introduction. The material compared gave the following result:

+++ corresponding to 50-70% living sperms (average: *ca.* 60%).
++ corresponding to 30-50% living sperms (average: *ca.* 40%).
+ corresponding to 10-30% living sperms (average: *ca.* 20%).

Over 70 per cent. living sperms was not demonstrated with this technique, and according to our experiences with Danish breeding bulls it would be of rather rare occurrence. The correlation demonstrated here holds true no matter whether the ejaculates are dense or less dense. Thus, if a specimen of semen contains 1 to 2 living sperms for each dead one, the dead sperms will be swept around in rapid " whirls," and on microscopy the semen will get the mark of +++. But, if each living sperm has to move about with 1 or 2 dead ones, the more sluggish type of motion will be seen; this is frequently encountered in bulls after a long period of abstinence. The first ejaculate will be very concentrated, though with a maximal motility of ++. In very thin ejaculates, with about 0.2 million sperms per c.mm., the wave motion will appear only very indistinctly.

In order to make the use of this simple method of estimating motility as consistent and uniform as possible, I have had a special slide made, which ensures that the layer of semen under examination will always be of the same thickness. This slide was designed primarily for motility estimation, but may be used, if necessary, for the other important microscopic examinations, namely, estimation of the concentration and of the movement of the individual sperms.

In order to carry out this microscopic examination of undiluted bull semen, three layers of semen are required, differing in thickness. The best motility estimation was obtained with a layer measuring 350 μ in thickness, this being the thinnest layer in which the wave motion proceeded unhampered. For estimation of the motility of individual sperms it was essential to have the layer of semen as thin as possible—and this was obtained best by placing the semen between two plane-ground, closely compressed slides. Finally, the density or concentration could be estimated most reliably in a layer of semen measuring 50 μ in thickness, this proving to be the thickness at which those differences in concentration that are of particular interest in judging bull semen were most distinctly pronounced.

An adjuvant which at once meets the requirement about these three thicknesses of layers is the contrivance I have constructed and called a comparing-chamber (Fig. 3).

As originally described,[2] and as shown in Fig. 3, it consists of a ground plate, *a*, measuring 3 mm. in thickness, on which are fastened a round glass disc, *b*, and a glass disc, *c*, with a large hole in the middle. When a plane-ground counting-chamber cover-slip, *d*, is mounted on top, 3 compartments, different in

height, are formed, namely: in the middle, the circular *central chamber*, with a diameter of 10 mm. and a height of 50 μ; encircling this, the *ring chamber*, measuring 2 mm. in width and 350 μ in height; and most peripherally the extremely low *outer chamber* (merely a few μ in height) between the two plane glass plates.

The 3 mm. thick ground plate has a considerable advantage over a slide of ordinary thickness, for, if necessary, the motility estimation may be carried out without employment of a heating-box round the microscope. With a little

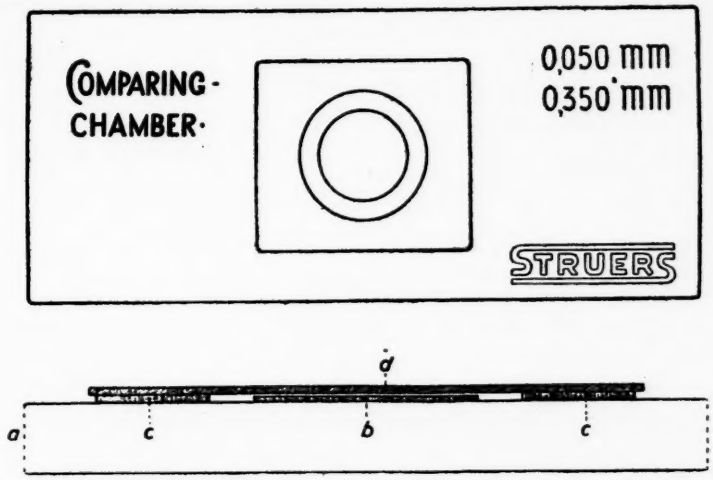

FIG. 3.

FIG. 3.—Comparing-chamber. Above: Chamber seen from above, natural size. Below: Drawing of chamber in longitudinal profile, enlarged 2.5 times. The chamber is manufactured by: *Leech Optical Co.*, Leicester, England, and distributed by *Struers chemiske Laboratorium*, Copenhagen.

experience it is sufficient simply to heat the filled comparing-chamber to body temperature over a steady flame of an alcohol burner, because the thick glass of the ground plate retains the heat fairly well, so that its temperature falls very slowly while the motility of the sperms is being estimated.

Employment of the Comparing-chamber

In employing the comparing-chamber, the examiner must keep in mind that this is a precision instrument that has to be handled with the same care as a counting-chamber. It should not be exposed to heating over 60° C., and every time it has been used it should be cleaned at once by means of tepid water.

When the chamber is to be used, it is first heated in the heating-box or over an alcohol burner to nearly body temperature (for control, the dorsal surface of the hand will serve as test object; all movements of the sperms cease on heating to 46-48° C.). Then, by means of a Pasteur pipette, or a glass rod, 3-4 drops (= about 1/10 c.c.) of the undiluted semen are distributed evenly in the central and ring chambers. The plane-ground cover-slip is pushed into place from the side, in order to avoid air-bubbles, making sure that it is pressed firmly against the underlying plate glass. During this procedure the outer chamber will generally be filled too.

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After cautious after-heating of the filled chamber, the three examinations under the microscope are carried out in the following succession:

	Magnification employed.
(1) Motility estimation	× 20- 50
(2) Estimation of the individual movements ...	× 300-400
(3) Estimation of the concentration	× 100

(1) The *motility estimation* is made in the ring chamber at low magnification (× 20-50), with employment of the diaphragm, after the scale of marks covering the following F. degrees, which I have been using in recent years (originally only 4°):

+++ signifies an excellent motility ("rapid whirls"). The wave and whirl formation is very pronounced; the features are changing very rapidly, because of the mass movements..

++(+)

++ signifies a fairly good motility ("sluggish waves"). The arrangement in darker waves or swarms is rather distinct, but the waves move slowly, the mass movement being sluggish.

+(+)

\+ characterises poor motility ("single propulsions"). Even on repeated after-heating it is not possible to induce any wave or whirl formation; among the evenly distributed sperms in the specimen, only a minority move about. With higher magnification many sperms may be seen to have quick and progressive propulsion.

(+) means only a few sperms moving about (< 10 per cent.); often in an abnormal way.

0 signifies necrospermia—no motile sperms whatever.

Such gradation of the findings requires some experience. Gradually, as one becomes more trained, however, it will be possible to employ also the intermediate marks: ++(+), +(+), and (+)—which will give a more accurate estimation of the material.

For normal breeding bulls that are being rationally used the motility degrees +++ and ++(+) will be by far the most common. In dense specimens the waves, as a rule, will be more distinct than in less concentrated ones.

In greatly used bulls the ejaculates will sometimes be rather thin, with apparently 90-100 per cent. of the sperms swarming about profusely, so that no regular waves are formed at all. Such specimens have to be marked with +++.

Sometimes the estimation of the motility may be rendered difficult because the wave motion is more or less checked by the admixture of tiny emulsified droplets of vaseline, originating from excessive greasing of the artificial vagina. Also an admixture of certain substitutes for vaseline (tylose slime) that were highly advertised during the war may affect the general features of the semen to such an extent as to preclude any worth-while microscopic examination. In some cases an admixture of pus or some other pathological secretion may completely abolish wave formation, which means that such specimens also will be rated "low" as far as their motility is concerned.

(2) *Estimation of the individual sperm movements* is performed at a magni-

FIG. 1.—Size 1/1.
Microphoto showing wave formation in the semen. Magnif. × 20.

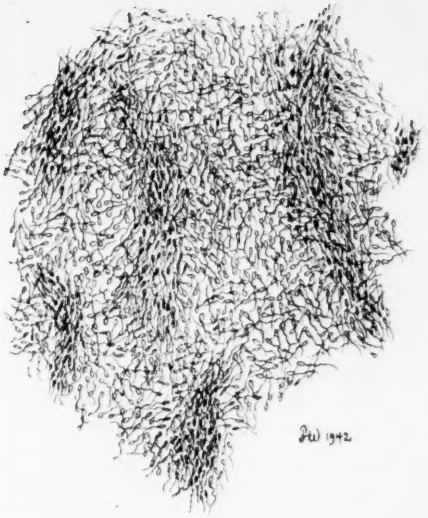

FIG. 2.—Size 1/1.
Drawing of beginning wave formation in a layer of semen, 100 μ in thickness. Magnif. × 200. (*Poul. H. Winther* pinx.)

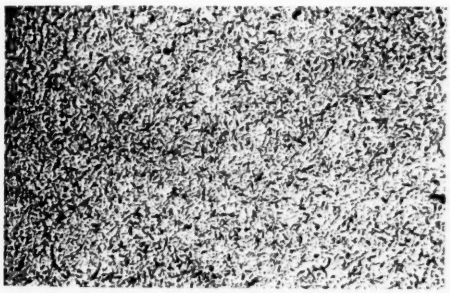

FIG. 4.—Size 1/1.
Microphoto of the lower border value (1.0 million per mm.^{3}) for the concentration degree *D—Sperma densum* in a layer of 50 μ. Magnif. × 100.

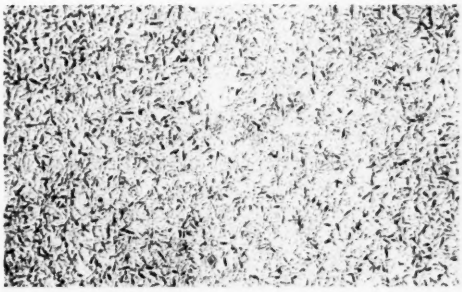

FIG. 5.—Size 1/1.
Microphoto of the lower border value (0.5 million per mm.^{3}) for the concentration degree *SD—Sperma semidensum* in a layer of 50 μ. Magnif. × 100.

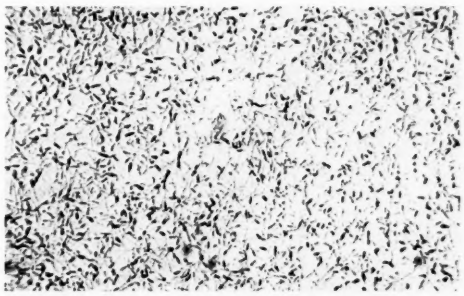

FIG. 6.—Size 1/1.
Microphoto of the lower border value (0.2 million per mm.^{3}) for the concentration degree *R—Sperma rarum* in a layer of 50 μ. Magnif. × 100.

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fication of about ×300 in the very thin layer of semen present in the outer chamber between the perforated glass plate *c* and the plane-ground cover-slip. This layer of semen is very suitable for this part of the examination, as the sperms will be sufficiently far apart without being inhibited in their movements. It can be noticed whether the progressive is the predominant form of movement, or whether, for instance, a great many of the sperms show motility anomalies (most often moving backwards or in circles, swinging from side to side, or with other abnormal movements), so that the general impression has to be summed up under the designation: oscillating movement.

Furthermore, this peripheral part of the chamber is also just as suitable as an ordinary thin cover-slip preparation for demonstration of gross abnormalities in semen—*e.g.*, admixture of pus cells, or an excessive number of detached sperm heads. The study of finer morphological details requires examination in an Indian ink preparation or a stained smear.

(3) *Estimation of the concentration* is performed after the glass has cooled, by microscopic examination of the layer in the central chamber at a magnification of ×100 and with marked narrowing of the diaphragm. Here it is to be emphasised that the lower the temperature of the specimen and, consequently, the less active the sperms, the more accurate is the estimation of the concentration. Experience has shown that employing a layer of semen, 50 μ in thickness, it is possible without any difficulty to distinguish between the concentrations: 1.0 million, 0.5 million and 0.2 million per c.mm. Thus we have a basis for the following scale of concentrations:

D = *Sperma densum*: 1.0 million sperms per c.mm., or more.
SD = *Sperma semidensum*: 0.5—1.0 million sperms per c.mm.
R = *Sperma rarum*: 0.2—0.5 million sperms per c.mm.
OS = *Oligospermia*: under 0.2 million sperms per c.mm.
A = *Aspermia*: complete absence of sperms.

In bulls that are being used rationally, *Sperma densum* ought to be the predominant degree of concentration. *Oligospermia* may be encountered in healthy bulls, but very seldom. *Aspermia* will practically never occur unless the specimen happens to result from an incomplete ejaculation (which may yield a secretion produced entirely by the accessory genital glands), or perhaps in cases of bilateral epididymitis. Even when both testes are attacked by an inflammatory process, the ejaculate will most often contain a few sperms or free sperm heads.

In order to employ the concentration scale here suggested, the examiner will have to be familiar with the appearance of the three concentrations—1.0, 0.5 and 0.2 million sperms per c.mm.—under the microscope. Microphotos of such standard preparations are shown in Figs. 4 to 6. The employment of similar photomicroscopic preparations for the gradation of samples under estimation would be the ideal procedure.

For further orientation, the following general description of the concentration degrees *D*, *SD* and *R* in a layer of semen 50 μ thick will be serviceable: in *Sperma semidensum* the sperm heads are separated by intervals of about the

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same size as the head. Finally, in *Sperma rarum* with an even distribution the intervals between the sperm heads will be larger than the sperm heads themselves (cf. Figs. 4—6).

The total result of the microscopic examination of the semen in the comparing-chamber, comprising the motility of the sperms, their "movement-type" and the concentration of the ejaculates may be entered schematically as shown in Fig. 7, which gives a very ready survey of the findings besides being convenient to use in the record of the bull. The outcome of the estimation may also be recorded as a coefficient, in which the degree of concentration is entered above the line of fraction, while the mark for motility and remark about movements are placed below this line. As, for instance: D/++(+) progress., OS/(+) oscill.

In such examinations the personal judgment will naturally still play some rôle. There can be no doubt, however, that by means of the comparing-chamber and a microscope with magnifications of about 50, 100 and 300 diameters it is possible to characterise the physiological quality of a given bull ejaculate within a few minutes in a way that will be far more satisfactory than the usual microscopic procedure. A further advantage is that this method requires only a relatively small amount of semen (3 drops = about 1/10 c.c.).

Fig. 7. *Schema for Recording of Semen.*

	D Sperma densum > 1.0 million/c.mm.	*SD* Sperma semidensum 0.5-1.0 million/c.mm.	*R* Sperma rarum 0.2-0.5 million/c.mm.	*OS* Oligospermia < 0.2 million/c.mm.
+++ 50-70% living sperms.				
++ 30-50% living sperms.	progressive			
+ 10-30% living sperms.				
0				

The principle of classification after the comparing-chamber method. Specimens of bull semen falling outside the rectangle framed by heavy lines will generally have to be characterised as unserviceable for artificial insemination. (Specimen D/++ progressive, is recorded here to illustrate the use of this *schema.*)

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Summary

A description is given of a new type of glass chamber suitable for the quick microscopic estimation of the quality of bull semen. By means of three different heights in this chamber, it is possible to carry out three different estimations of the given specimen with one preparation:

Motility is estimated at body temperature in a layer of 350 μ in thickness, the estimation being based on the wave formation produced by the mass movements of the sperms, and especially by the degree of rapidity of the wave motion.

The type of *movement of individual sperm* is examined in the very thin layer of semen formed between two plane-ground glass plates pressed together.

The *concentration* is estimated in a layer of semen measuring 50 μ in thickness.

The gradation of the specimen is made by comparison with standard concentrations of *1.0, 0.5* and *0.2* million sperms per c.mm.

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THE TREATMENT OF PURULENT CONJUNCTIVITIS IN DOMESTICATED ANIMALS WITH A MIXED SULPHANILAMIDE-SULPHATHIAZOLE POWDER:

A note on the experimental treatment of two cases

By MAHOMED FOUAD SAID, D.V.M.S.(Egypt),
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THE discovery of the sulphonamide drugs has created a new type of chemotherapy in human and veterinary medicine, and a vast literature now exists on the sulphonamide treatment of bacterial infections, mainly of streptococcal, pneumococcal, and staphylococcal types in human and animal subjects. Since the original introduction of "Prontosil rubrum" (sulphonamido-crysoidin), a great variety of sulphonamides have been prepared, and among these the following have been tested and found to give good results: sulphanilamide, sulphathiazole, sulphapyridine, sulphadiazine, sulphaguanidine, sulphasuxidine, sulphamezathine, sulphamerazine, sulphacetamide, etc.

These drugs exhibit quantitative differences in bacteriostatic power, solubility, and rate of absorption from the intestine. This allows a certain amount of freedom in the selection of the drug most suitable for the type of infection and circumstances under which treatment is to be applied. In general, however, sulphanilamide tends to be effective only against streptococci, sulphapyridine against streptococci and pneumococci, and sulphathiazole and sulphadiazine against streptococci, pneumococci, and staphylococci. The slow rate of absorption of sulphaguanidine and sulphasuxidine makes them especially useful for the treatment of intestinal infections, and their value for this purpose in

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veterinary medicine has been well demonstrated (e.g., calf scours, feline and canine dysentery). It is also of interest here to mention the work of Horton Smith and E. L. Taylor (1942), who reported good results with sulphamethazine in the treatment of coccidiosis in chickens.

Naturally, on account of the wide use and great value of sulphonamide drugs in internal medicine, considerable attention has been given to their pharmacological and toxicological properties, particularly in human beings. In animals, however, the toxic effects of these drugs are less common and less important than they are in human beings, according to Bourne (1944) and also Krill (1944).

Since 1939 the sulphonamides have been used in the form of ointments, creams, pastes and powders for external prophylaxis and treatment of infections. Sulphanilamide alone or in mixture with sulphathiazole has been recommended and widely used for the control of infections in wounds and burns and to prevent the systemic spread of infections from the injured sites. The same two sulphonamides in powder form have also been much used to reduce the risk of infection in exposed tissues during surgical operations on human beings and animals. It is recognised that the bacteriostatic action of the sulphonamides is inhibited by pus and necrotic tissue, and that this may explain the contrary reports on the effectiveness of sulphonamides in the control of infections, particularly in deep and freely suppurating wounds.

In human medicine the local application of sulphonamides for superficial infections of the skin has fallen out of favour, owing to the risk of sensitisation, which may lead to the development of an intractable dermatitis or to an acute sensitivity to sunlight. The chances of sensitisation, however, are negligible if the drug is applied for not more than five days. Moreover, there is some evidence that the same risk is also reduced if the sulphonamides are combined with zinc oxide (or carbonates).

Menton (1945) has successfully treated purulent conjunctivitis in human being with an eye-paste composed of sulphapyridine 20 per cent., cod-liver oil 40 per cent., petroleum jelly 40 per cent., coupled with the internal administration of sulphapyridine and sulphathiazole. Robson and Scott (1944) experimented with various sulphonamide drugs, particularly sodium sulphacetamide, in the form of eye-drops and ointments (2.5-30 per cent.) for the treatment of streptococcal infections artificially induced in the eye of the rabbit, and concluded that the local treatment was more effective than systemic administration.

Experimental

The author has experimented with the direct application of powdered sulphonamides in cases of purulent conjunctivitis in animals which proved to be very resistant to the usual methods of treatment, and observations on two such cases are now reported.

Case No. 1

A horse suffering from purulent conjunctivitis of the right eye, associated with a copious flow of discharge, deeply injected conjunctiva, and œdematously swollen eye-lids.

Treatment was conducted according to general rules for a couple of days, and

which included the use of antiseptic compressors, antiseptic eye-washes, eye-drops, etc., without apparent successful results, except slight signs of improvement.

Microscopical examination of the conjunctival discharge revealed mixed coccal infection.

As a result of the failure of this treatment, sulphanilamide and sulphathiazole were put into trial in the form of a powder mixture with other drugs for the treatment; this mixture is as follows :—

℞

Pulv. sulphanilamide,	1 part.
Pulv. sulphathiazole,	1 part.
Pulv. zinc oxide (pure),	2 parts.
Pulv. amylum,	1 part.

This sulphonamide mixture can be easily applied into the eye by either the aid of a powder blower or by any other means.

Previous to the dusting process, the unaffected eye was first washed well with 2 per cent. tepid solution of sodium bicarbonate, and thoroughly dried with clean cotton-wool. This treatment was carried out twice daily, once in the morning and the other in the afternoon.

Case No. 2

An alsatian dog suffering from the same trouble, showed the same outstandings symptoms of the affection as described in case No. 1, and from the start it was treated by the same sulphonamide mixture.

Results

In the clinical cases already mentioned the trial of the above formula proved to be superior to the usual modes of treatment in cases of purulent conjunctivitis in domesticated animals.

In case No. 1 improvement in the condition showed itself after the lapse of 48 hours in the gradual decrease of the ocular discharge, the subsidence of the inflammation, and the return of the eye to normal condition in a period of 7 days.

In case No. 2 the same good results were secured in a week's time.

It is interesting to record here that the same sulphonamide mixture was used in a similar affection in a girl with definite result in an interval of 5 days, using the same mode of treatment.

Discussion

The rapid cures secured by this type of medication clearly demonstrated the superiority of the given formula over drops, ointments, and the internal administration of sulphonamide drugs. It may be suggested that the favourable results obtained were due to the following factors :—

(1) The high bacteriostatic power of sulphanilamide and sulphathiazole combined.

(2) The removal of pus material by sodium bicarbonate wash, thereby promoting the maximum effect of the drugs.

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(3) The direct application of the mixture in contact with the infection in its powder form.

(4) The astringent effect of zinc oxide in the mixture in reducing the production of the discharge.

Acknowledgment

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NOTES ON THE SHOEING OF CEYLON BULLS BY ITINERANT FARRIERS

By L. A. P. BRITO-BABAPULLE, B.Sc., M.R.C.V.S., D.T.V.M.

VETERINARY literature is rather scanty in information about shoeing of bulls. This article aims at filling that gap. The draught animal in Ceylon and other Eastern countries is commonly the bull, at times the male buffalo, sometimes the ass and mule, and only very rarely the horse. The camel and the elephant are the unshod draught animals. On soft, sodden ground, as encountered in the agricultural processes of ploughing, harrowing, etc., the wear and tear on the hoof horn is almost negligible when compared to that encountered by cart-bulls drawing heavy loads on hard macadamised roads. The wasting off of the hoof horn can be so excessive as to " come to the quick " in four days of gruelling work.

Anatomy.—The claws of the bovine, four in number, cover the ends of the digits in each limb. The chief digits, two in number in each limb, may be considered as the working digits and are the ones that are shod. For purposes of description there are three surfaces to each claw:

(1) The abaxial surface or wall of the claw.

(2) The interdigital surface.

(3) The basal or ground surface.

This surface, in conjunction with its fellow, presents a ground surface which, from the internal side to the external, can be termed as made up of the following edges: The medial, medio-internal, (interdigital), medio-external (interdigital), and lateral edges. The nails and nail-holes are solely restricted to the medial and lateral edges. Clips have very occasionally been used on the medio-external and medio-internal edges, respectively, in splay-footed animals.

Tools Employed.—The photograph gives a pictorial idea of the tools used.

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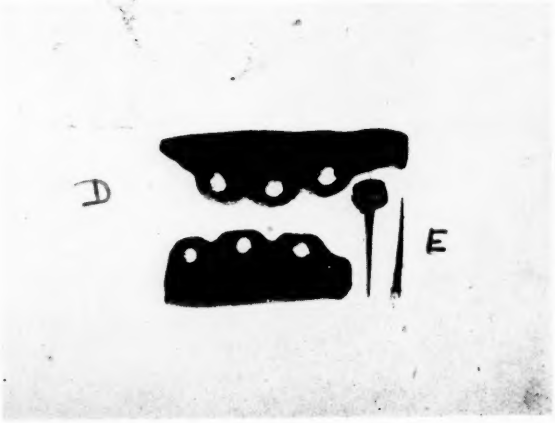
KEY TO ILLUSTRATIONS:

A—Hoof surface of shoe, with clip.
B—Ground surface of shoe, with clip.
C—Flat shoe, without clips.
D—Worn-out shoes.
E—Nails, flat and side views.



From the right to the left there are: (1) Casting rope of pliable hemp. (2) A portable anvil. Sometimes this takes the shape (unlike the illustration) of a 10″ long, 1″ thick, poker-shaped nail with a flat head, total weight 1 lb. This is conveniently set up by driving into the ground. It is also used for tightening the clenches. (3) A sharp-pointed awl for boring nail-holes in the hoof. (4) Toeing-knife, usually triangular-shaped, with a flat head, one of the angles for percussion with a hammer for paring down the hoof. (5) Pincers. (6) A punch for punching nail-holes in the shoe. (7) Shoeing hammers, in between which lie shoe-nails, and (8) a small toeing-knife used for cutting seats for the clenches when counter-sunk into the wall. (9) Tray for carrying the tools, along one edge of which lies (10) a piece of timber, ½″ thick, 2″ wide, and 1½′ long. Above this piece of wood is seen (11) a small metal vessel containing coconut-oil (oleum cocos nucifera), which contains astringent bark, herbs, etc., incorporated in it by heat and trituration, presenting a glutinous mass. (12) Shoes shaped to conform to the basal surface of each claw. They are made usually of wrought iron, and are cast in moulds. Very rarely are they forged at time of shoeing. The shoes are usually flat, and many of them are provided with toe-tips, ¾″ long and ¼″ square, because the cart-bull is notorious for dragging its toes at a slow pace. The nail-holes are 3 to 5 in number, depending on the size of the shoe; usually it has 3 holes, which are left seated but not perforated or punched until just before use. (13) A file-headed rasp (not shown), and (14) a bolster of wrapped sacking.

Preparation.—Casting is done by one rope round the loins of the bull, which is tightened by a squeeze-knot, and as the animal sinks to the ground the feet are secured together and the squeeze-rope relaxed. The feet are then propped up on the bolster.

Technique.—The clenches are knocked up, using the smaller toeing-knife, the pincers are then applied between the shoe and the horn and the horn is levered off the basal surface. Any nail wasted out flat and that works itself through the worn nail-hole is pulled off with the pincers. The hoof is then wiped clean with a dry cloth. The overgrown horn is then pared down, using the toeing-knife, hammer, and rasped flat. The piece of plank is used to gauge the flatness of the basal surface. A suitable shoe is then placed, and any unevenness is hammered out on the portable anvil, and the shoe made to fit the hoof. Nail-holes are then punched out, and with a cold fit-on the point of entry of the nail is mapped out on the dry basal surface with the awl dipped in the astringent oil. The shoe is removed and the nail-hole bored with the awl in a direction upwards and outwards in relation to the recumbent animal. The shoes are placed on the basal surface and the nails placed in situ, with the anvil head as a medium to turn down the clenches. In the forelegs the nail-spikes are clipped off close to the hoof with the pincers and then hammered into position in little depressions cut for them on the abaxial surface of the claw. In the hind legs the spike is hammered and rolled into a coil, which is flattened into depressions in the wall. The ropes are removed and the animal allowed to stand.

The nail-heads stand out like frost-nails in horse-shoeing, but soon wear down smooth.

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CLINICAL ARTICLE

CASE REPORT: RABIES IN A HEIFER

By CALVIN F. BENNETT

Corry, Penn.

SUBJECT: Guernsey heifer, approximately 18 months old, in excellent condition.

Symptoms: Profuse sweating and steaming like a horse suffering with an acute attack of azoturia; temperature 103.4° F., accelerated breathing, upright posture, with nose intermittently pressed down hard on the floor; mouth soiled with fæces and dirt. Because of the frequency with which the nose was pressed on the floor it was becoming raw and bleeding. A blanket placed on the animal was immediately grabbed in her mouth, pulled off and pressed by her nose against the floor. Any lighter material was immediately pulled off. Sometimes the material was shaken from side to side, then pressed on the floor. After a minute or so she would seem to lose her equilibrium, partially fall, and after regaining her balance, raised her nose. When one approached her, she would attempt to face the individual, particularly if something was waved in front of her. If one made an attempt as if to strike her in the face she would never dodge, but would seem to come into the blow without fear. Intermittently when the owner would leave the stable she would look fixedly at the door from which he left and bellow. The voice had an off-normal sound, but not extreme. She frequently dribbled small quantities of urine, and her bowel movements, after several normal defæcations, finally became a nearly transparent, gelatinous mucus. When the writer feinted to attack her, she rushed at him as far as her tie would permit and bellowed savagely. Extreme weakness developed and the animal would frequently fall, remaining in a recumbent position for about one-half minute before arising. Death occurred the next day.

The *clinical diagnosis* was rabies.

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